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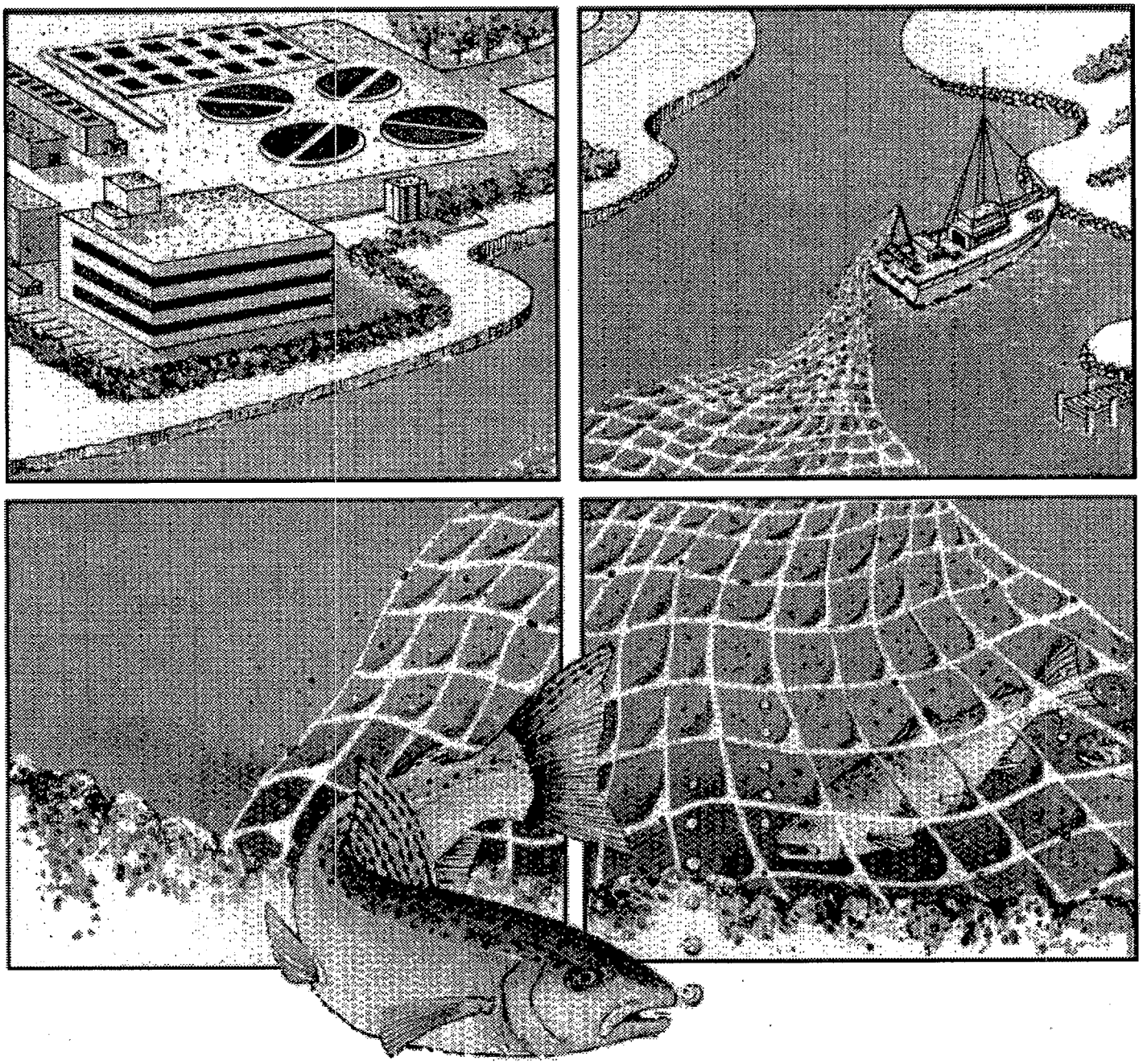

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CWA SECTION 403: PROCEDURAL AND MONITORING GUIDANCE

March 1994

United States Environmental Protection Agency
Office of Wetlands, Oceans and Watersheds
Oceans and Coastal Protection Division
Washington, DC

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ABBREVIATIONS

BOD	-	Biochemical oxygen demand
COE	-	United States Army Corps of Engineers
CWA	-	Clean Water Act
CZMA	-	Coastal Zone Management Act
CZMP	-	Coastal Zone Management Plan
EIS	-	Environmental Impact Statement
EPA	-	United States Environmental Protection Agency
MGD	-	Million gallons per day
MMS	-	Minerals Management Service
NESDIS	-	National Environmental Satellite, Data and Information Service
NMFS	-	National Marine Fisheries Service
NPDES	-	National Pollutant Discharge Elimination System
NRC	-	National Research Council
NOAA	-	National Oceanic and Atmospheric Administration
NWI	-	National Wetlands Inventory
ODCE	-	Ocean Discharge Criteria Evaluation
OWEC	-	Office of Wastewater Enforcement and Compliance
PCS	-	Permit Compliance System
POTW	-	Publicly-owned treatment works

GLOSSARY

Acute - A stimulus severe enough to rapidly induce an effect; in aquatic toxicity tests, an effect observed in 96 hours or less typically is considered acute. When referring to aquatic toxicology or human health, an acute effect is not always measured in terms of lethality.

Acute-to-chronic ratio (ACR) - The ratio of the acute toxicity of an effluent or a toxicant to its chronic toxicity. ACR is used as a factor for estimating chronic toxicity on the basis of acute toxicity data, or for estimating acute toxicity on the basis of chronic toxicity data.

Alternatives assessment - An assessment of the relative impacts (economic, social, and environmental) of on-site effluent discharge versus discharge to an alternative site, land disposal, or another waste disposal alternative.

Ambient - Of or relating to a condition of the environment, such as temperature or pressure, that surrounds a body or object.

Anadromous fish - Fish that spend their adult life in the sea but swim upriver to freshwater spawning grounds to reproduce.

Analyte - The substance being identified and measured in an analysis.

Anoxic - Lack of oxygen.

Anthropogenic - Caused or influenced by the activities of humans.

Baseline - Defines the landward boundary of the territorial seas.

Benthic - Of, relating to, or occurring at the bottom of a waterbody.

Benthic biota - A form of aquatic plant or animal life that is found on or near the bottom of a body of water.

Bioaccumulation - Process by which a compound is taken up by an aquatic organism, both from water and through food.

Bioassay - A test used to evaluate the relative potency of a chemical or a mixture of chemicals by comparing its effect on a living organism with the effect of a standard preparation on the same type of organism.

Biocriteria - Narrative expressions or numeric values of the biological characteristics of aquatic communities based on appropriate reference conditions.

Biochemical oxygen demand (BOD) - A measure of the amount of oxygen consumed in the biological processes that break down organic matter in water. The greater the BOD, the greater the degree of pollution.

Biogeographical - The geographical distribution of animals and plants.

Biomass - A quantitative estimate of the entire assemblage of living organisms considered collectively and measured in terms of mass volume or energy in calories.

Calibration - The determination of any equipment deviation from a standard source so as to ascertain the proper correction factors.

Chronic - A stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. Chronic should be considered a relative term depending on the life span of an organism. The measurement of a chronic effect can be reduced growth, reduced reproduction, etc., in addition to lethality.

Coastal zone - Lands and waters adjacent to the coast that exert an influence on the uses of the sea and its ecology or, inversely, whose uses and ecology are affected by the sea.

Compensation depth - The depth at which there is just enough light to allow the amount of oxygen produced by phytoplankton through photosynthesis to balance the amount consumed by their respiration.

Composite sample - A single effluent sample collected over a 24-hour period, on which only one toxicity test is performed.

Contiguous zone - Defined in section 502(9) of the Clean Water Act to be "the entire zone established or to be established by the United States under Article 24 of the Convention of the Territorial Sea and the Contiguous Zone."

CTD instruments - Sampling instruments that measure conductivity, temperature, and depth.

Cytogenetic - Referring to chromosomal aberrations.

Data Quality Objectives - Specific, integrated statements and goals developed for each data or information collection activity to ensure that the data are of the required quantity and quality.

Drogue - A small object attached to a surface buoy to measure currents.

Effect concentration - A point estimate of the toxicant concentration that would cause an observable adverse effect (such as death, immobilization, or serious incapacitation) in a given percentage of the test organisms.

Effluent - Wastewater—treated or untreated—that flows out of a treatment plant, sewer, or industrial outfall. Generally refers to wastes discharged into surface waters.

Effluent limitation - Any restriction on quantities, rates, or concentrations of chemical, physical, biological, and other constituents that are discharged from point sources into waters of the United States, including navigable waters of the contiguous zone or the ocean.

Epifaunal - Living in the surface of the sediment.

Euphotic zone - The upper layers of a lake or ocean into which sufficient sunlight penetrates for photosynthesis (usually down to about 80 meters).

Fecal coliform bacteria - Bacteria found in the intestinal tracts of animals. They are an indicator of possible pathogenic contamination.

Grab samples - Samples collected over a very short period of time and on a relatively infrequent basis. A separate toxicity test must be performed on each grab sample.

Habitat - The place where a population lives and its surroundings, both living and nonliving.

Hard-bottom habitats - Those benthic habitats such as coral reefs, rocky subtidal areas, and "live bottom" sponge/gorgonian habitats.

Hermatypic coral - Reef-building coral.

Hypoxic conditions - A deficiency of oxygen.

Indicator species - A species whose characteristics show the presence of specific environmental conditions.

Indigenous species - A species native to or occurring naturally in a particular region or environment.

Industrial source - For the purpose of this report, a nonmunicipal source of wastewater discharges.

Infaunal - Living in the sediment.

Irreparable harm - Significant undesirable effects occurring after the date of permit issuance that will not be reversed after cessation or modification of the discharge. (40 CFR 125.121(a))

In situ - In the normal or natural position.

Lipids - Fats or fat-like substances that contain aliphatic hydrocarbons, are water insoluble, and are easily stored in the body for use as fuel.

Lowest-Observed-Adverse-Effect Level (LOAEL) - The lowest concentration of an effluent or toxicant that results in statistically significant adverse health effects as observed in chronic or subchronic human epidemiology studies or animal exposure.

Marine waters - Territorial seas, the contiguous zone, and the oceans.

Mixing zone - An area where an effluent discharge undergoes initial dilution and is extended to cover the secondary mixing in the ambient waterbody. A mixing zone is an allocated impact zone where water quality criteria can be exceeded as long as a number of provisions are maintained.

Municipal source - A public source of wastewater discharges.

National Pollutant Discharge Elimination System (NPDES) - The national permitting program for issuing, modifying, revoking and reissuing, terminating, monitoring, and enforcing permits, and imposing and enforcing pretreatment requirements, under sections 301, 307, 318, 402, 403, and 405 of the Clean Water Act.

No-Observed-Adverse-Effect Level (NOAEL) - A tested dose of an effluent or a toxicant below which no adverse biological effects are observed, as identified from chronic or subchronic human epidemiology studies or animal exposure studies.

No-Observed-Effect Concentration (NOEC) - The highest tested concentration of an effluent or a toxicant at which no adverse effects are observed on the aquatic test organisms at a specific time of observation. Determined using hypothesis testing.

Ocean Discharge Criteria - 40 CFR Part 125, Subpart M, which establishes guidelines for issuance of an NPDES permit for the discharge of pollutants from a point source into the territorial seas, the contiguous zone, and the oceans.

Ocean Discharge Guidelines - Ten narrative guidelines listed at 40 CFR 125.122 of the Ocean Discharge Criteria regulations for determination of unreasonable degradation of the marine environment.

Ocean Discharge Factors - Seven narrative factors listed at section 403(c)(1)(A)-(G) of the Clean Water Act for determination of the degradation of the marine environment.

Pathogens - Microorganisms that can cause disease in other organisms or in humans, animals, and plants.

Phytoplankton - A type of plant plankton that is the basic source of food in many aquatic and marine ecosystems.

Plankton - A collective term for the wide variety of plant and animal organisms, often microscopic in size, that float or drift freely in the water because they have little or no ability to determine their own movement.

Pretreatment - The reduction of the amount of pollutants, the elimination of pollutants, or the alteration of the nature of pollutant properties in wastewater prior to or in lieu of discharging or otherwise introducing such pollutants into a POTW. The

reduction or alteration may be obtained by physical, chemical, or biological processes, by process changes, or by other means, except as prohibited by 40 CFR Part 403.

Publicly-owned treatment works (POTW) - A treatment works, as defined in section 212(2) of the Clean Water Act, that is owned by a State, municipality, or intermunicipal or interstate agency.

Pycnocline - The stratifying vertical density gradient in the water column caused by differences in temperature and/or salinity.

Sediment quality criteria - Elements of sediment quality standards, expressed as constituent concentrations, levels, or narrative statements representing a quality of sediment that supports a particular use. Sediment quality criteria reflect the use of available scientific data to assess the likelihood of significant environmental effects on benthic organisms from chemicals in the sediment and to derive regulatory requirements that will protect against these effects.

Soft-bottom habitats - Those benthic habitats composed of a significant benthic biological community consisting of organisms that burrow into the substratum.

Spatial - Of or relating to space; involving relations or measurements that have to do with space.

Stormwater discharge - Precipitation that does not infiltrate the ground or evaporate because of impervious land surfaces but instead flows onto adjacent land or water areas and is routed into drain/sewer systems.

Stressed waters - Waters that have been impaired by pollutants.

Technology-based treatment requirements - NPDES permit requirements based on the application of pollution treatment or control technologies including (under 40 CFR Part 125) best practicable technology (BPT), best conventional technology and secondary treatment for POTWs (BCT), best available technology economically achievable (BAT), and new source performance standards (NSPS).

Temporal - Of or relating to time; involving relationships or measurements that have to do with time.

Territorial seas - Defined in section 502(8) of the Clean Water Act to be the belt of the seas measured from the line of ordinary low water along that portion of the coast which is in direct contact with the open sea and the line marking the seaward limit of inland waters, and extending seaward a distance of 3 miles.

Toxic - Harmful to living organisms.

Toxicity characterization - A determination of the specific chemicals responsible for effluent toxicity.

Toxicity test - A procedure to determine the toxicity of a chemical or an effluent using living organisms. A toxicity test measures the degree of effect on exposed test organisms of a specific chemical or effluent.

Unreasonable degradation - Significant adverse changes in ecosystem diversity, productivity, and stability of the biological community within the area of discharge and surrounding biological communities; threat to human health through direct exposure to pollutants or through consumption of exposed aquatic organisms; loss of aesthetic, recreational, scientific, or economic value that is unreasonable in relation to the benefit derived from the discharge. (40 CFR 125.121(e))

Volatile organic compounds (VOCs) - Any organic compound that participates in atmospheric photochemical reactions.

Water quality-based toxics control - An integrated strategy used in NPDES permitting to assess and control the discharge of toxic pollutants to surface waters: the whole-effluent approach involving the use of toxicity tests to measure discharge toxicity and the chemical-specific approach involving the use of water quality criteria or State standards to limit specific toxic pollutants directly.

Water quality criteria - Elements of State water quality standards, expressed as constituent concentrations, levels, or narrative statements, representing a quality of water that supports a particular use. When criteria are met, water quality will generally protect the designated use.

Water quality standards - Provisions of State or Federal law that consist of a designated use or uses for the waters of the United States and water quality criteria for such waters based on such uses. Water quality standards are to protect the public health or welfare, enhance the quality of water, and serve the purposes of the Clean Water Act.

Whole-effluent toxicity - The aggregate toxic effect of an effluent measured directly by a toxicity test.

Zone of initial dilution (ZID) - The region of initial mixing surrounding or adjacent to the end of an outfall pipe or diffuser ports.

Zooplankton - A type of animal plankton.

EXECUTIVE SUMMARY

THE PURPOSE AND IMPLEMENTATION OF THE 403 PROGRAM

The Clean Water Act (CWA, or the Act), Public Law 95-217, was enacted in 1972. Throughout the years it has been periodically amended, with the most extensive amendments being adopted in 1977 and the most recent in 1987. The Act is the single most important and comprehensive piece of legislation dealing with the environmental quality of the Nation's waters, covering both marine and freshwater systems.

Section 402 of the CWA established the National Pollutant Discharge Elimination System (NPDES). This section of the Act requires that any direct discharger of pollutants to the surface waters of the United States obtain an NPDES permit before the discharge can take place. To obtain an NPDES permit, a discharger must demonstrate compliance with all applicable requirements of the Act. In the case of discharges to the territorial sea, the contiguous zone, or the ocean,[1] these requirements include section 403 of the Clean Water Act.

Section 402 permits are intended to control the release of pollutants into waters of the United States from point source discharges such as municipal and industrial outfalls. some stormwater discharges are also covered. Dischargers are required to meet the Act's minimum technology-based treatment requirements. Discharges to State waters are also required to comply with State water quality standards. In addition to these requirements, discharges to marine waters are also subject to section 403 of the Clean Water Act, which sets forth criteria to prevent unreasonable degradation of the marine environment and authorizes imposition of any additional effluent limitations, including zero discharge, necessary to protect the receiving waters to attain the objectives of the Clean Water Act.

To implement section 403, EPA has established 10 ocean discharge guidelines that, along with other provisions of 40 CFR Part 125, Subpart M, provide the basis for determining whether a discharge will cause unreasonable degradation of the marine environment. Under the regulations, if it is determined that a discharge would cause unreasonable degradation, the permit will be denied; discharges that will not cause unreasonable degradation may be permitted and the permit may include conditions necessary to ensure that unreasonable degradation will not occur during the time of the permit. These determinations are made using available information, including

[1] For ease of reference, the waters of the territorial sea, contiguous zone, and oceans will hereinafter be referred to as "marine waters."

information provided by the permit applicant, relevant environmental impact statements, section 301(h) or other variance applications, existing technical and environmental field studies, and EPA industrial and municipal waste surveys.

In those cases where there is insufficient information to determine whether unreasonable degradation will occur, under 40 CFR 125.123(a) and (b), a permit will not be issued unless (1) it can be determined that no irreparable harm will result from the discharge, (2) there are no reasonable alternatives to the discharge, and (3) the discharge will comply with certain permit conditions specified in the regulations. These conditions include bioassay-based discharge limitations and monitoring to assist in determining whether and to what extent further limitations are necessary to ensure that the discharge does not cause unreasonable degradation.

Because of the case-specific nature of permit decision making, the review and extent of required monitoring associated with NPDES permits issued under the provisions of section 403 are variable. This is due to the wide range of receiving environments and the variability of the discharge. This document provides guidance on determining the nature and extent of monitoring studies needed to assess the impact of the discharges on the marine environment.

USE OF THIS DOCUMENT

This document is designed to provide the EPA Regions and NPDES-authorized States with a framework for the decision-making process for section 403 evaluations and to provide guidance on the type and level of monitoring that should be required as part of permit issuance under the "no irreparable harm" provisions of section 403. (Generally, ambient monitoring is not required if a determination of "no unreasonable degradation" is made.) The decision-making aspects of the program, such as determination of information requirements and sufficiency of information, determination of no unreasonable degradation, and the decision to issue/reissue or deny a permit, are described. Options for monitoring under the basis of no irreparable harm, including criteria for evaluating perceived potential impact and establishing monitoring requirements to assess actual impacts, are discussed. Finally, summaries of monitoring methods for evaluating the following parameters are provided:

- *Physical characteristics*, such as temperature, salinity, density, depth, turbidity, and current velocity and direction, to characterize the water column, to verify hydrodynamic models, and to indicate spatial and temporal variations;
- *Water chemistry* to evaluate the quality of receiving waters;
- *Sediment chemistry* to determine pollutant levels in sediments;
- *Sediment grain size* to describe spatial and temporal changes in the benthic community;

- *Benthic community structure* to detect and describe spatial and temporal changes in community structure and function;
- *Fish and shellfish pathobiology* to provide information regarding damage or alteration to organ systems of fish and shellfish;
- *Fish and shellfish populations* to detect and describe spatial and temporal changes in the abundance, structure, and function of fish and shellfish communities;
- *Plankton characteristics* including biomass, productivity, and community structure and function, to identify the dominant species, detect short- and long-term spatial and temporal trends, and examine the relationship between water quality conditions and community characteristics;
- *Habitat identification* to determine whether pollutant-related damage will cause long-lasting harm to sensitive marine habitats;
- *Bioaccumulation* to provide the link between pollutant exposure and effects;
- *Pathogens* to assess water conditions in the vicinity of discharges and surrounding areas and to assess relative pathogen contributions from permitted effluent discharges;
- *Effluent characterization* to predict biological impacts of an effluent prior to discharge; and
- *Mesocosms and microcosms* to assess ecological impacts from marine discharges.

Each method section contains an explanation of why the measurement of the parameter of concern might be included as part of a 403 monitoring program, and a discussion of monitoring design considerations, analytical methods, statistical design considerations, the use of data generated, and quality assurance/quality control considerations.

1. INTRODUCTION

The Clean Water Act (CWA, or the Act), Public Law 95-217, was enacted in 1972. Throughout the years it has been periodically amended, with the most extensive amendments being adopted in 1977 and the most recent in 1987. The Act is the single most important and comprehensive piece of legislation dealing with the environmental quality of the Nation's waters, covering both marine and freshwater systems.

Section 402 of the CWA established the National Pollutant Discharge Elimination System (NPDES). This section of the Act requires that any direct discharger of pollutants to the surface waters of the United States obtain an NPDES permit before the discharge can take place. To obtain an NPDES permit, a discharger must demonstrate compliance with all applicable requirements of the Act, including section 403 (for marine discharges). Section 402 permits are intended to control the release of pollutants into the navigable waters of the United States from all point sources, including municipal, industrial, and some stormwater discharges.

The regulatory framework established under section 402 provides a two-pronged approach for controlling point source discharges. The first, reliance on "technology-based" standards, consists of national minimum treatment requirements based on an assessment of the achievability of control technologies by individual categories of dischargers. The second, a "water quality-based" approach, stresses water quality criteria, water quality standards, and the setting of pollutant effluent limitations intended to maintain receiving surface water quality at a level sufficient to protect classified designated uses of the receiving waterbody (e.g., fishable/swimmable).

Discharges to marine waters are subject to additional regulatory requirements established under section 403 of the CWA. Section 403 applies to marine discharges under NPDES permits and allows for more stringent controls when necessary to protect the environment. It is not restricted by engineering attainability, nor is it limited by rigorous cost or economic restrictions when determining permit conditions. It includes consideration of sediment as well as water column effects. It not only protects aquatic species but also places special emphasis on unique, sensitive, or ecologically critical species. Under section 403, EPA or an NPDES-authorized State can impose discharge limitations or other conditions needed to attain compliance with section 403.

The Ocean Discharge Criteria implementing section 403 are intended to prevent unreasonable degradation of the marine environment and to authorize imposition of effluent limitations, if necessary, to achieve this goal. The Ocean Discharge Criteria were promulgated on October 3, 1980, 45 FR 65942 (see Appendix B). The Ocean

Discharge Criteria provide flexibility for permit writers to customize permit application requirements, effluent limitations, and reporting requirements to the specific conditions of each individual discharger's situation in order to ensure compliance with section 403. The criteria also ensure consistency by imposing minimum requirements in situations where the long-term impacts of the discharge are not fully understood.

1.1 THE OCEAN DISCHARGE CRITERIA

Section 403(c)(1) of the Clean Water Act specifies seven factors that are to be included in guidelines for determining the potential for degradation of marine waters. These seven factors are the foundation for the 10 ocean discharge guidelines contained in the EPA regulations and discussed in Section 2.2.3 of this document. Under these 10 guidelines and the other provisions of the Ocean Discharge Criteria, no NPDES permit may be issued that will cause unreasonable degradation of the marine environment. Prior to permit issuance, the director (defined as the EPA Regional Administrator or the State Director where there is an NPDES-authorized State program, or an authorized representative) is required to evaluate whether a proposed discharge will cause such degradation. To make this determination, the director considers the provisions specified in 40 CFR 125.122(a) and (b).

In cases where sufficient information is available for the director to make a determination whether unreasonable degradation of the marine environment will occur, the director is governed by 40 CFR 125.123(a) and (b). Discharges that cause unreasonable degradation are prohibited; other discharges may be permitted under conditions necessary to ensure that unreasonable degradation will not occur during the term of the permit.

In cases where the director is unable to determine whether unreasonable degradation will occur, 40 CFR 125.123(c) applies. Under this provision no discharge will be allowed unless the director can determine that:

- No irreparable harm will result from the discharge;
- There are no reasonable alternatives to the discharge; and
- The discharge will comply with certain permit conditions, including bioassay-based discharge limitations and monitoring requirements. These conditions will assist in determining whether and to what extent further limitations are necessary to ensure that the discharge does not cause unreasonable degradaton. Pursuant to 40 CFR 125.123(d)(4), if on the basis of new data the director determines that continued discharges may cause unreasonable degradation of the marine environment, the permit must be modified or revoked.

The Ocean Discharge Criteria encourage the use of any available information, in addition to information supplied by the permit applicant, in determining the degradation of marine waters as a result of a discharge. Therefore, the director may make a

determination using information contained in relevant environmental impact statements, section 301(h) or other variance applications, existing technical and environmental field studies, or EPA industrial and municipal waste surveys.

1.2 PURPOSE OF THIS DOCUMENT

Because of the difficulty and uncertainty inherent in predicting the precise impacts of discharges on marine waters, ocean discharge permits may be issued/reissued under the "no irreparable harm" provisions of 40 CFR 125.123(c) and, as a result, will require monitoring studies to more precisely assess the impact of these discharges on the marine environment. The purpose of this document is to provide the Regions and NPDES-authorized States with a framework for the decision-making process to be followed in making a section 403 determination and to provide them with guidance for identifying the type and level of monitoring that should be required as part of a permit issued under the "no irreparable harm" provisions of section 403.

1.3 DOCUMENT FORMAT

Chapter 2 of this document presents an explanation of, and procedural guidance for, the overall process to be followed when issuing an NPDES permit in compliance with section 403 of the Clean Water Act. Chapter 3 discusses options for monitoring under the basis of "no irreparable harm." Chapter 4 presents a summary of monitoring methods with potential applications to 403 discharges.

2. SECTION 403 PROCEDURE

2.1 BACKGROUND

2.1.1 The Role of the Ocean Discharge Criteria in NPDES Permit Issuance

The Ocean Discharge Criteria (40 CFR Part 125, Subpart M) are used in the development of NPDES permits for the discharge of pollutants into marine waters. The criteria are designed to protect marine resources from the impact of wastewater discharges and to prevent unreasonable degradation of the marine environment. Although referred to as the Ocean Discharge Criteria, they are promulgated regulations that establish minimum requirements on discharges to marine waters. The 10 guidelines presented in the Ocean Discharge Criteria that must be considered when reviewing a permit request under section 403 are presented in Table 2-1.

The regulations promulgated pursuant to section 403 are not a substitute for other authorities of the Clean Water Act, but are a component of section 402 NPDES permits and apply *in addition to* other applicable Clean Water Act requirements. Permittees subject to section 403 must still comply with all other provisions of the Act including sections 301 (technology-based effluent limitations), 303 (water quality standards), 306 (national standards of performance), and 307 (pretreatment). Permittees also may be subject to section 311 (oil and hazardous substances) and section 312 (marine sanitation devices).

The NPDES program is administered by EPA's Office of Wastewater Enforcement and Compliance (OWEC); the section 403 program is administered by the Office of Wetlands, Oceans and Watersheds (OWOW). OWOW is responsible for developing the criteria and guidelines for ocean discharges that are subject to section 403. OWEC is responsible for administering the NPDES permitting program. The actual NPDES permits are issued by EPA Regional offices or, in States that have been granted NPDES permitting authority, by the designated State environmental agency.

2.1.2 Applicability of Section 403

Point source discharges to waters of the United States are subject to the requirement to obtain a section 402 NPDES permit. Permits for discharges seaward of the baseline of the territorial seas must comply with the requirements of section 403. The "baseline" typically follows the line of ordinary low water along the portion of the coast that is in direct contact with the open sea although closing lines may be drawn straight across the mouths of bays, in certain cases.

Table 2-1. Ocean Discharge Guidelines

(1) Quantities, composition, and potential bioaccumulation or persistence of the pollutants to be discharged;

(2) Potential transport of the pollutants by biological, physical, or chemical processes;

(3) Composition and vulnerability of potentially exposed biological communities, including

- unique species or communities,
- endangered or threatened species, and
- species critical to the structure or function of the ecosystem;

(4) Importance of the receiving water area to the surrounding biological community, e.g.,

- spawning sites,
- nursery/forage areas,
- migratory pathways, and
- areas necessary for critical life stages/functions of an organism;

(5) The existence of special aquatic sites, including (but not limited to)

- marine santuaries/refuges,
- parks,
- monuments,
- national seashores,
- wilderness areas, and
- coral reefs/seagrass beds;

(6) Potential direct or indirect impacts on human health;

(7) Existing or potential recreational and commercial fishing;

(8) Any applicable requirements of an approved Coastal Zone Management Plan (CZMP);

(9) Such other factors relating to the effects of the discharge as may be appropriate; and

(10) Marine water quality criteria.

Section 403 requirements apply only to point source discharges beyond the baseline of the territorial sea (represented in Figure 2-1 with a heavy black line). This limits the number of land-based discharges subject to section 403. For example, only the discharges numbered 3 through 9 in Figure 2-1 would be subject to the provisions of section 403.

EPA's permit regulations (40 CFR 122.21) require that permit applicants list the latitude, longitude, and name of the receiving waters for each outfall. Where the permitting authority is uncertain as to whether the outfall is within the waters covered by section 403, guidance should be requested from EPA Headquarters. In some waters where the baseline location is unsettled, e.g., where the coastline is highly irregular, EPA will contact the Department of State, which chairs an interagency group responsible for defining the boundaries of the territorial seas.

2.1.3 Individual or General Permit

Most NPDES permits are issued to individual facilities with one or more outfalls. A number of conditions exist, however, that may warrant the issuance of a "general permit" covering multiple sources (40 CFR 122.28). These conditions include the following:

- When there are expected to be a number of the same or substantially similar discharges;

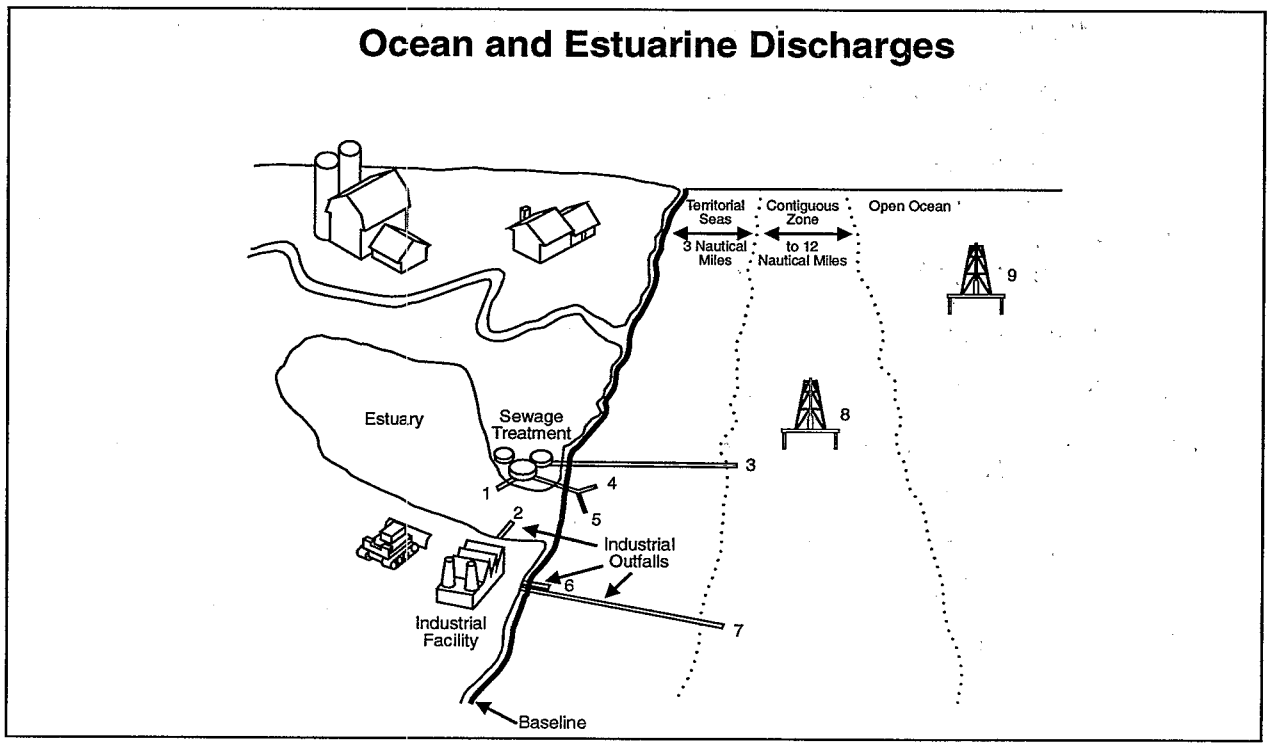


Figure 2-1. Applicability of Section 403 Requirements

- When such discharges are expected to have the same effluent limitation requirements; and
- When, in the opinion of the director, the discharges would be more appropriately controlled by a general permit.

General permits may be written only if the area receiving the effluent is of a homogenous nature; if it is circumscribed by the same geographical or political boundary; if the uses of the receiving waters are of similar value; and if the area is not significantly distorted by oceanographic anomalies. Examples of discharges covered under a general permit include offshore oil and gas operations and seafood processing operations. Exceptions to provisions of a general permit can be controlled as subcategories within the permit as long as distinguishing features of the subcategory are easily identified (e.g., water depth, lease block numbers).

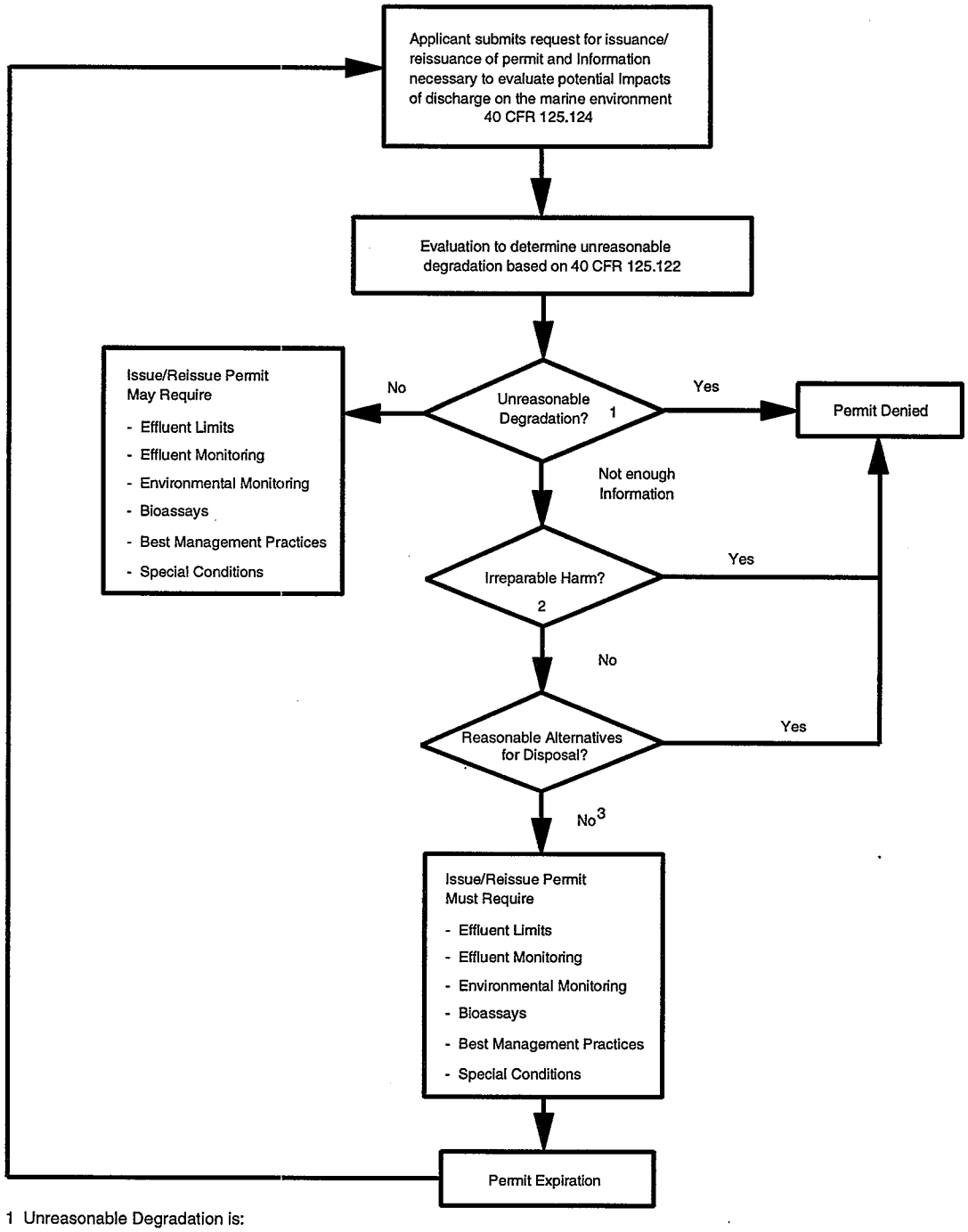
Dischargers requesting coverage under a general permit must only notify EPA of their intent to be covered and provide the necessary information to establish similarity to the permitted discharges. In writing a general permit, however, EPA is the permit applicant and must fulfill all the necessary data requirements.

2.2 GENERAL PROCEDURE

The discussion that follows presents guidance on the procedure to be followed by permit writers when reviewing an NPDES permit request under section 403 and deciding whether to issue such a permit and under what conditions. Once a determination has been made that section 403 applies to a particular discharge, a general progression of decisions is required to complete an Ocean Discharge Criteria Evaluation (ODCE). Figure 2-2 presents the decision process that is undertaken in the review of a section 402 permit request involving ocean discharges. The discussion that follows describes each of the steps in this process. Much of this discussion is also presented in the preamble to the section 403 regulations (45 FR 65942, October 3, 1980).

2.2.1 Request for Issuance/Reissuance of a Section 402 Permit

Any point source discharger to the navigable waters of the United States that meets the criteria defined in section 402 of the Clean Water Act must obtain a section 402 NPDES permit prior to discharging. In addition, any permit issued under section 402 for discharges to marine waters must also comply with the requirements of section 403. A permit application is submitted to the State in which the discharge occurs if the State has been granted NPDES permitting authority by EPA. EPA processes the permit application if the State in which the discharge occurs is not authorized or if the discharge occurs in Federal waters (waters beyond the territorial seas).



1 Unreasonable Degradation is:
(1) Significant adverse changes in ecosystem diversity, productivity and stability of the biological community within the area of discharge and surrounding biological communities.
(2) Threat to human health through direct exposure to pollutants or through consumption of exposed aquatic organisms; or
(3) Loss of aesthetic, recreational, scientific, or economic values which is unreasonable in relation to benefit derived from the discharge.

2 Irreparable harm is significant undesirable effects which will not be reversed after cessation or modification of the discharge.

3 Assuming other applicable requirements are met.

Figure 2-2. Section 403 Decision Process

2.2.2 Determination of Information Requirements

Prior to the issuance of an NPDES permit for a discharge subject to section 403, the director must determine that "no unreasonable degradation" of the marine environment will occur as a result of the discharge. Such a determination is to be made after consideration of the effects of pollutant discharge on human health and welfare, marine life, and aesthetic, recreational, and commercial values; the persistence and permanence of these effects; the effects of varying disposal rates; the alternative disposal or recycling options available; and the effect on alternative uses of the ocean.

Before any decision regarding the degradation of the marine environment can be made, the director must first determine what information is necessary to evaluate the effects of the discharge based on the guidelines presented in the Ocean Discharge Criteria. The director should survey the currently available information about the discharge and the area in which the discharge would occur. This information would include the data contained in the permit application form, as well as data available from other Agency reports and studies.

Following a survey of currently available information, the director should determine what additional information might be required from the applicant for the ODCE under 40 CFR 125.124. The permitting authority can require the discharger to supply information necessary to make a determination of "no unreasonable degradation," including the following:

- Analysis of chemical constituents of the discharge;
- Bioassays necessary to determine limiting permissible concentrations for the discharge;
- Analysis of initial dilution;
- Process modifications that will reduce the quantities of pollutants that will be discharged;
- Analysis of the locations where pollutants are sought to be discharged, including biological communities, and physical description of the discharge facility; and
- Evaluation of available alternatives to the discharge of pollutants, such as land-based disposal.

The applicant is responsible for collecting the additional information and submitting it to the director for review.

2.2.3 Determination of No Unreasonable Degradation

The section 403 regulations define unreasonable degradation as

> . . . significant adverse changes in ecosystem diversity, productivity and stability of the biological community within the area of discharge and surrounding biological communities, threat to human health through direct exposure to pollutants or through consumption of exposed aquatic organisms, or loss of aesthetic, recreational, scientific or economic values which is unreasonable in relation to the benefit derived from the discharge.

The determination of no unreasonable degradation is to be made based on a consideration of the 10 guidelines in 40 CFR 125.122. Discussion of these guidelines also appears in the preamble to the section 403 regulations (45 FR 65942, October 3, 1980). The following discussion is intended to provide basic technical guidance that might be used in determining compliance with the section 403 criteria.

(1) Quantities, composition, and potential bioaccumulation or persistence of pollutants to be discharged. An assessment of the potential effects of a discharge on the marine environment should carefully consider the potential presence of persistent or toxic pollutants, especially when present in significant quantities relative to marine water quality criteria developed pursuant to CWA section 304(a). The potential for bioaccumulation or persistence of pollutants in the environment is of particular importance. In areas that do not contain sensitive species or unusual biological communities or are not important for surrounding biological communities, discharges containing primarily conventional pollutants may be of lesser concern if data indicate that there will be significant mixing with the receiving waters based on the flow of the discharge and the physical characteristics of the discharge site, such as water depth, density gradient, and turbulence (USEPA, 1980). An assessment of the effluent characteristics should include the types, sources, amounts, and temporal characteristics of the effluent, as well as its physical, chemical, and toxicological properties and known or demonstrated environmental impacts.

(2) Potential transport of the pollutants by biological, physical, or chemical processes. An assessment of receiving water characteristics important to the transport and fate of pollutants should include an evaluation of the physical, chemical, hydrodynamic, and sedimentologic features of the waterbody. An assessment of the biological processes that may impact the fate and transport of contaminants and their daughter products also may be necessary and would include consideration of degradation through physicochemical processes, metabolic processes, bioturbation, and potential for transport through the food chain.

(3) Composition and vulnerability of potentially exposed biological communities. An assessment should be made of the presence of, and potential for, effects of a discharge on the species and biological communities that might be impacted by the discharge. Particular attention should be given to the possible presence of species identified as endangered or threatened pursuant to the Endangered Species Act, and species critical to the structure or function of the ecosystem, such as species in food chain relationships.

(4) Importance of the receiving water area to the surrounding biological community. The permitting authority should consider the vulnerability of the area of discharge and its role in the larger biological community. Environmentally significant or sensitive areas such as spawning sites, nursery or forage areas, migratory pathways or areas necessary for other functions or critical stages in the life cycles of organisms, areas of high productivity, or areas under stress due to biological or climatic conditions or discharges from other sources would be of most concern.

(5) Existence of special aquatic site. The potential for impacts on special aquatic sites near or adjacent to the discharge should be considered. Special aquatic sites include both those of special biological significance and those of aesthetic or recreational importance. Examples presented in the Ocean Discharge Criteria include:

- Marine sanctuaries,
- Refuges,
- Parks,
- National and historic monuments,
- National seashores,
- Wilderness areas, and
- Coral reefs.

(6) Potential direct or indirect impacts on human health. Under the 403 criteria, the permitting authority is to consider the potential impacts of the discharge on human health, either directly through physical contact or indirectly through the food chain. The location of the discharge, the type and volume of the discharge's effluent, and the nature of the surrounding biological communities should all be considered in performing this assessment.

(7) Existing or potential recreational and commercial fishing. In determining whether unreasonable degradation will occur, the 403 crtieria call for consideration of potential impacts on recreational and

commercial fishing. This will involve identification of existing and potential fishery resources that might be adversely affected by the discharge and consideration of the nature and extent of these effects.

(8) Any applicable requirements of an approved Coastal Zone Management Plan (CZMP). The Ocean Discharge Criteria specifically identify the need to assess the applicable requirements of an approved State Coastal Zone Management Plan. Once a State has an approved Coastal Zone Management Plan, the Federal consistency provisions of section 307(c)(3) of the Coastal Zone Management Act (CZMA) generally require that applicants for Federal licenses or permits obtain a certification from the State as to consistency with the approved plan. This obligation applies to NPDES permits (see 40 CFR 122.49(d)).

(9) Such other factors relating to the effects of the discharge as may be appropriate. Permitting authorities are given the prerogative to evaluate factors other than those specifically defined in the Ocean Discharge Criteria when making a determination of "no unreasonable degradation." These factors could include other statutory requirements, impacts on other uses of the ocean such as navigation or resource exploitation, or potential biological impacts not previously described. The permitting authority should determine what additional information is appropriate under given discharge circumstances and request such information from the applicant.

(10) Marine water quality criteria. Discharges to the territorial seas are subject to and must comply with any applicable State water quality standards. In the absence of such State standards, or in waters beyond a State's jurisdiction, EPA marine water quality standards developed under section 304(a) of the CWA should be considered when evaluating discharges. Although not binding like State water quality standards under section 303, the EPA section 304 marine water quality standards provide important technical information that should be considered in performing an ODCE.

Following a review of existing information related to the 10 guidelines described above, the permitting authority prepares an ODCE to compile and explain the information base, conclusions, and regulatory determinations and requirements related to a section 403 review. The ODCE should specifically address each of the 10 guidelines set out in 40 CFR 125.122 and, in general, may be organized under five broad categories of information:

(1) Characterization of the effluent and receiving water

- discharge,
- receiving water,
- fate and transport;

(2) Potential effects

- aquatic life,
- human health,
- socioeconomic;

(3) Other statutory/regulatory factors

- CZMA,
- Endangered Species Act,
- others (State regulations, treaties, administrative directives, etc.);

(4) Findings/Conclusions; and

(5) Regulatory outcome or actions.

A copy of the ODCE should be made available to NPDES permitting staff for inclusion as part of the Administrative Record for the associated NPDES permit. One of three determinations is possible after an evaluation of a discharge based on a review and assessment of the Ocean Discharge Criteria as presented in an ODCE: (1) unreasonable degradation will not occur; (2) unreasonable degradation will occur; or (3) insufficient information is available to make a determination.

2.2.4 Decision to Issue/Reissue or Deny a Permit

A determination of "no unreasonable degradation" during the permit application review process will allow the permit development and issuance process to proceed. The permit may or may not impose further data-gathering requirements (i.e., monitoring), discharge limitations, or other special conditions deemed necessary to prevent any future unreasonable degradation of the marine environment. It may also include a reopener clause. In practice, virtually all permits subject to section 403 have included reopener clauses. If during the permit application review process a determination is made that unreasonable degradation of the marine environment will occur, the permit will be denied (40 CFR 125.122(b)).

2.2.5 Insufficient Information

In those cases where there is insufficient information to make a determination of "no unreasonable degradation," under 40 CFR 125.123(c) the discharge cannot be permitted unless three criteria are met:

(1) No irreparable harm will result from the discharge;

(2) There are no reasonable alternatives to onsite disposal; and

(3) The discharge will comply with certain mandatory permit conditions necessary to ensure that unreasonable degradation will not occur.

No Irreparable Harm

The first determination that must be made following a decision of "insufficient information" is that the discharge will not cause irreparable harm to the marine environment during the period for which the discharge will be permitted.

The Ocean Discharge Criteria define irreparable harm as "significant undesirable effects occurring after the date of permit issuance which will not be reversed after cessation or modification of the discharge." In evaluating the potential for irreparable harm, the permitting authority will need to make a determination of whether the discharger, operating pursuant to its permit conditions, will not cause permanent and significant harm to the environment during the period in which further data on the effects of the discharge are collected. Certain factors are particularly significant in assessing the likelihood of irreparable harm, including the quantity of pollutants expected to be discharged and their potential for persistence in the marine environment. An additional factor is the sensitivity of the area into which the discharge is proposed. For example, a discharge could cause irreparable harm to unusual and interdependent communities, such as coral reefs and associated communities. Data on the effects of similar discharges in similar areas are directly relevant to the determination of irreparable harm. Information demonstrating the timely recovery of the environment after the cessation of discharges from similar facilities would be a strong indication that irreparable harm is not likely to occur (USEPA, 1980). In addition to considering the permanence of the impacts, consideration must also be given to the significance of the anticipated impacts. This would involve consideration of such factors as the areal extent of impacts, the ecological or economic significance of affected resources, and potential impacts on human health.

Reasonable Alternatives

If it is determined that no irreparable harm will occur as a result of a discharge, then the second determination that must be made prior to permitting the discharge is that there are no reasonable alternatives (as defined in 40 CFR 125.121(d)) to on-site disposal. Such alternative sites would include disposal facilities located on land and discharge points within internal waters. In determining whether a site is a reasonable alternative to on-site disposal, the permitting authority should consider its distance from the location of the proposed discharge and whether its use would cause unwarranted economic impact on the discharger. The amount of material requiring disposal should also be considered along with the availability of existing land-based disposal sites within a reasonable distance from the point of discharge and the estimated uncommitted capacity of such sites. A second basis for evaluating the feasibility of alternative sites is the relative environmental harm of disposal associated with each alternative. Alternative sites are not considered "reasonable alternatives" if on-site disposal is judged to be environmentally preferable.

Other Mandatory Permit Conditions

The final criterion for authorizing discharges for which there is insufficient information to make a determination of "no unreasonable degradation" is that the discharge be in compliance with all permit conditions established pursuant to §125.123(d) of the regulations, including a bioassay-based discharge limitation (similar to those of EPA's ocean dumping regulations, 40 CFR Part 227) and monitoring requirements. These permit conditions are specified to assist in determining whether and to what extent further limitations are necessary to ensure that the discharge does not cause unreasonable degradation (USEPA, 1980).

Monitoring is required as part of a permit issued under the "no irreparable harm" provisions of section 403. One of the principal purposes of the monitoring program is to collect sufficient information to determine whether unreasonable degradation will occur as a result of the discharge. Such information is then used to make a decision whether to reissue the permit during the next round of permitting. In addition, it is vital that the monitoring program should also be designed to ensure that no irreparable harm to the marine environment will occur during the life of the current permit. In order to do this, the monitoring program that is put in place must be sufficient to assess the impact of the discharge on water, sediment, and biological quality and should include, where appropriate, analysis of the bioaccumulative and/or persistent impact on aquatic life. This monitoring program may include effluent analysis, bioassay analysis, and field studies (USEPA, 1980).

It is not possible to make an a priori determination as to what constitutes an acceptable and cost-effective monitoring program. Site-specific conditions such as the volume of waste discharged, the types of pollutants discharged, and the location of the discharge will play a role in determining what monitoring will be required of a discharger. These considerations are discussed further in Chapter 3 of this document.

In addition to bioassay and monitoring requirements, the permitting authority may also specify other permit conditions under 40 CFR 125.123(d). Seasonal restrictions on the volume of wastes discharged can be required where such restrictions are needed to ensure protection of the marine environment. Bioaccumulation testing of the liquid and/or suspended particulate phase of the discharge can be required where the potential for bioaccumulation exists, based on the nature of the pollutants discharged. Process modifications, such as the substitution of less hazardous chemicals for those that are potentially harmful, can be required, as can process changes that would favor the recycling and reuse of potentially harmful pollutants.

If the data gathered pursuant to 40 CFR 125.123(d), in particular the monitoring data, indicate that continued discharge may cause unreasonable degradation of the marine environment, under 40 CFR 125.123(d) the permit will be revoked or modified to include additional limitations as necessary.

3. OPTIONS FOR MONITORING UNDER THE BASIS OF "NO IRREPARABLE HARM"

3.1 BACKGROUND

The purpose of this chapter is to provide guidance to the EPA Regions and NPDES-authorized States for determining the general types and level of monitoring that should be required as part of a 402 permit issued or reissued under the section 403 provisions of "no irreparable harm." The guidance presented in this chapter is intended to be used as a general assessment tool. The determination of the specific monitoring requirements needed for a particular discharge involves a case-by-case determination based on the potential environmental threats posed by the particular discharge and consideration of existing information concerning site-specific conditions. Particular consideration should be given to the proximity of a discharge to sensitive ecological zones, the existence of known observed biological stress based on available baseline data for the area, discharges that have high mass emission rates and/or concentrations of priority pollutants and other toxic substances, and the presence of other discharges in the vicinity of the discharge in question.

A summary of methods available for conducting monitoring programs for various environmental parameters is presented in Chapter 4. This methods summary is presented as a guide for determining what methods are available or are being developed. The discussion that follows in this chapter identifies only the general types of monitoring that might be appropriate as part of a permit issued under the "no irreparable harm" provisions of section 403. The actual monitoring requirements specified in a 402 permit should be determined based on site-specific environmental and discharge conditions. The references cited in Appendix A, as well as other sources, should be referred to in the actual development and implementation of monitoring programs.

As pointed out by the National Research Council (NRC, 1990), despite the considerable efforts and expenditures associated with environmental monitoring, most programs fail to provide the information needed to understand the condition of the marine environment or to assess the effects of human activity on it. In its report *Managing Troubled Waters*, the NRC (1990) identified several factors critical to the design and implementation of an effective monitoring program. EPA suggests that the recommendations made by the NRC be incorporated into any monitoring program required under section 403. These recommendations include the following:

- The goals and objectives of the monitoring program need to be clearly articulated in terms that pose questions that are meaningful to the public and that provide the basis for scientific investigation.

- Not only must data be gathered, but attention must also be paid to their management, synthesis, interpretation, and analysis.
- Procedures for quality assurance are needed, including scientific peer review.
- Because a well-designed monitoring program results in unanswered questions about environmental processes or human impacts, supportive research should be provided.
- Adequate resources are needed not only for data collection but also for detailed analysis and evaluation over the long term.
- Programs should be sufficiently flexible to allow for their modification where changes in conditions or new information suggests the need.
- Provision should be made to ensure that monitoring information is made available to all interested parties in a form that is useful to them.

The central elements involved in designing and implementing an effective monitoring program, as presented in the NRC's report (1990), are illustrated in Figure 3-1.

3.2 CRITERIA FOR EVALUATING THE POTENTIAL FOR ENVIRONMENTAL IMPACT

To assess the potential impact posed by a discharge and thus the types and level of monitoring that might be necessary as part of a permit issued under the "no irreparable harm" provisions of section 403, the following site-specific determinations should be made:

- Is the discharge in question a "major" or a "minor" discharge?
- Does the discharge occur in stressed waters?
- Does the discharge occur in the vicinity of sensitive biological areas?
- What are the number and types of other discharges in the vicinity of the discharge in question?

3.2.1 Major/Minor Discharges

It is recommended that the Office of Wastewater Enforcement and Compliance's (OWEC) classification of major/minor discharges be used for according such status to discharges of concern. OWEC classifies industrial discharges as "major" or "minor" based on an evaluation of the potential for toxic pollutant discharge, traditional pollutants in the effluent, potential human health impacts, flow rate of effluent, and various water quality factors. Municipal discharges are classified as "major" if ownership is public, the facility is active, the flow rate is 1 million or more gallons per day or a population of 10,000 is served, or the discharge causes significant water quality impacts. In addition, each EPA Region, in consultation with the environmental agency of an NPDES-authorized State, is allowed to designate a certain percentage of its total

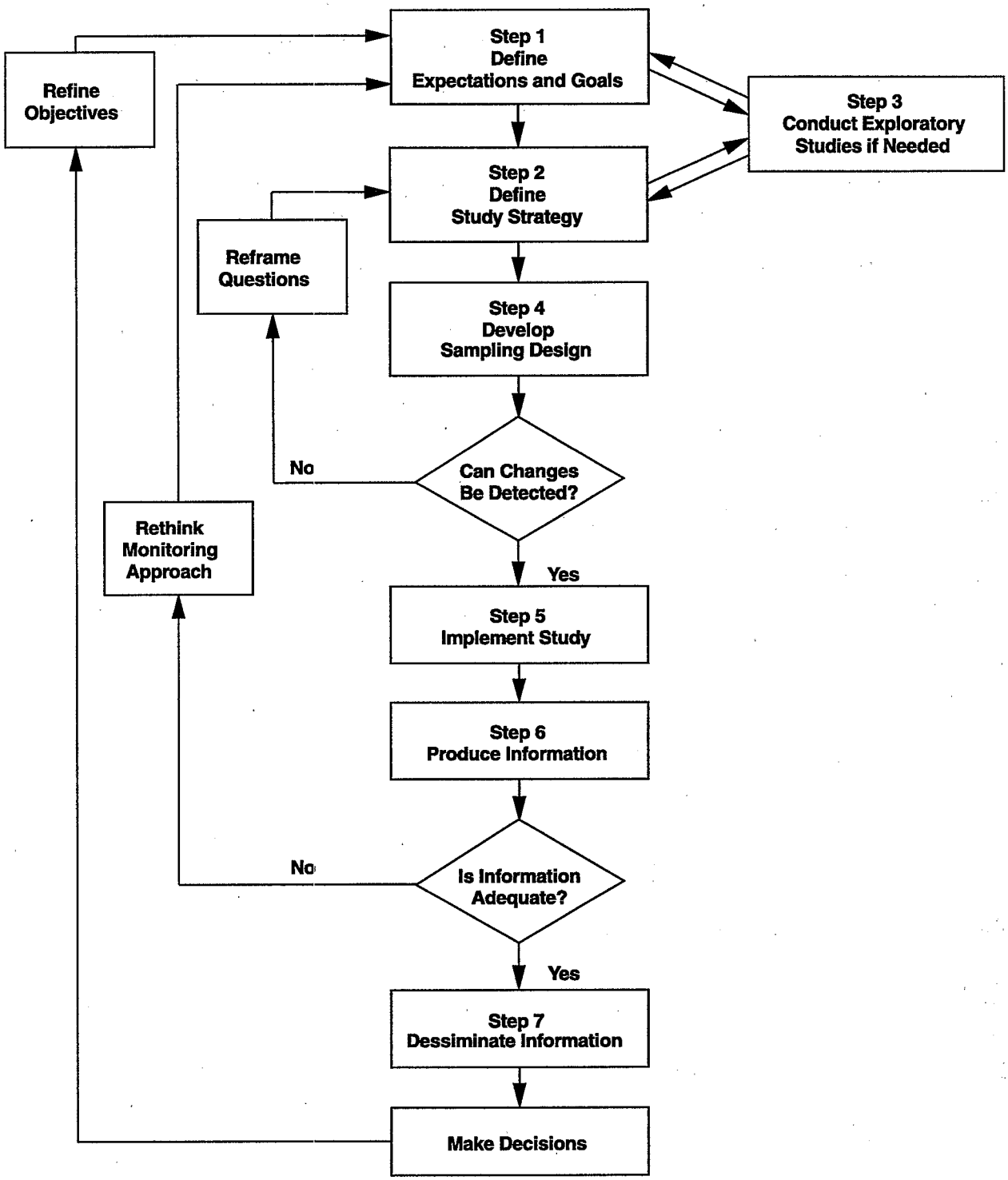


Figure 3-1. Elements of Designing and Implementing a Monitoring Program (NRC, 1990)

permits as "discretionary addition" major permits. This classification has been created for those permits that the Region or State believes should be accorded major status, but for some reason were not classified as such by OWEC.

OWEC's Permit Compliance System (PCS) is a data base management system that tracks individual facility permit conditions. The PCS Permit Facility Data File includes the major/minor classification for NPDES discharges.

3.2.2 Discharges to Stressed Waters

As part of the permit development process, a determination of whether a discharge occurs in or near stressed waters should be made, and the presence of stressed waters should be considered when determining the type and level of ambient monitoring that should be required of a discharger. Such ambient monitoring should be designed to determine whether and to what extent the permitted discharge is contributing to stressed conditions and whether permit limitations are helping to alleviate such stress.

Two sources of information can be used to assess whether a discharge of concern is occurring in stressed waters: State 305(b) reports and 304(l) lists.

Section 305(b) of the Clean Water Act requires States to report to EPA on the extent to which their surface waters are meeting the goals of the Act and to recommend how the goals can be achieved. Each State, territory, and Interstate Commission develops a program to monitor the quality of its surface water and groundwater and to report the current status of water quality to EPA in biennial Water Quality Assessment reports. Among other things, these 305(b) reports allow EPA to:

- Determine the status of water quality;
- Identify water quality problems and trends;
- Evaluate the causes of poor water quality and the relative contributions of pollution sources; and
- Determine the effectiveness of control programs.

In 1990, 11 States and two territories reported on the degree to which their coastal waters support the uses for which they have been designated. This represented 4,230 coastal miles or only 22 percent of the Nation's estimated 19,200 miles of coastline. Efforts are being made, however, to improve the 305(b) reporting process. These improvements hopefully will result in a higher percentage of States reporting on their coastal water quality conditions.

Another source of information on stressed coastal waters is the State 304(l) "long list." This is a comprehensive list of waters of the State that are impaired by point or nonpoint source discharges of toxic, conventional, and nonconventional pollutants. Although section 304(l) of the Clean Water Act was identified as a one-time listing of waters, States are encouraged to maintain updated information on the status of their near coastal waters. Unfortunately, as was the case with 305(b) reports, not all coastal States have assessed the condition of their offshore waters.

For those coastal waters that have not been assessed by the States, a determination of whether a discharge occurs in stressed waters should be made based on the best professional judgment of the permitting authority using other sources of existing information. Sources of additional information on potentially stressed waters include EPA's 301(h) and National Estuary programs, the Environmental Monitoring and Assessment Program (EMAP), and the National Oceanic and Atmospheric Administration's (NOAA) Strategic Assessment Program, as well as other biological/ecological assessments conducted by the Federal Government (e.g., Minerals Management Service (MMS) and U.S. Geological Survey (USGS) of the U.S. Department of the Interior, and the U.S. Army Corps of Engineers (COE)) and State governments.

3.2.3 Discharges to Sensitive Biological Areas

The determination of what constitutes a sensitive biological area is subject to the discretion of the permitting authority, but at a minimum should include the following:

- Spawning sites,
- Nursery areas,
- Migratory pathways,
- Marine sanctuaries/refuges,
- Coral reefs/seagrass beds, and
- Other areas necessary for critical life stages/functions of important marine organisms.

Numerous sources of information concerning the location of sensitive marine environments are available. These include previous surveys conducted by State or Federal environmental agencies. In addition to specific State environmental agencies, major Federal sources of such information include NOAA and the U.S. Fish and Wildlife Service (FWS) and MMS of the U.S. Department of the Interior. As part of the information-gathering effort for an ODCE conducted for a 403 review, the discharger should be required to investigate the existence of applicable information on sensitive

marine environments developed by these and other sources. Table 3-1 lists various sources in NOAA, FWS, and MMS for information regarding sensitive marine environments.

Several programs within NOAA compile information on important and sensitive marine habitats. NOAA's National Environmental Satellite, Data and Information Service (NESDIS) maintains an archive of worldwide data on the physical and chemical properties of the ocean. Over the past decade, NESDIS has received large amounts of physical, chemical, and biological data collected within the U.S. Exclusive Economic Zone. These data derive primarily from programs organized to study the effects of offshore oil development, ocean dumping, and other human activities on marine ecosystems. NOAA's National Marine Sanctuary program has the responsibility of preserving and restoring the conservation, recreational, ecological, or aesthetic values of designated marine sanctuaries. The program presently manages seven national marine sanctuary sites. The National Marine Fisheries Service (NMFS) of NOAA can provide information concerning the presence of spawning and nursery sites for important commercial and recreational fisheries, as well as information on catch of particular species in State and Federal offshore waters. NMFS also has responsibility for the management of some marine mammals under the Marine Mammal Protection Act of 1972; the FWS has management responsibility for others, including endangered species. Together, these two agencies have compiled considerable information on marine mammal populations, life cycles, and habitat requirements.

The FWS collects and interprets diverse information on fish and wildlife species, populations, and habitats. Included under the management responsibility of the FWS are anadromous fish species and endangered species, as well as some marine mammals. The FWS publishes ecological characterization studies and species profiles for numerous marine/estuarine fishery species. The FWS is also responsible for producing detailed wetland maps for the contiguous United States. The mapping of coastal wetlands has been an ongoing effort of the National Wetlands Inventory (NWI) program at FWS. Other habitat types, including submerged aquatic vegetation, rocky shores, reefs, and intertidal softbottoms, are also depicted on NWI maps although they are not covered as completely as the wetlands.

As part of its offshore oil and gas leasing program, the MMS has conducted numerous environmental impact studies and other special studies to assess the environment and potential impact of offshore oil and gas activities on marine resources in leasing areas. These studies include in-depth evaluations of the lease area, the affected environment, and the potential environmental consequences of offshore oil and gas activities.

Table 3-1. Information Sources on Sensitive Marine and Coastal Environments: NOAA, FWS, and MMS

NOAA

Strategic Environmental
Assessment Division
U.S. Department of Commerce
6001 Executive Boulevard
Rockville, MD 20852-3806
301/443-8843

Marine and Estuarine Management Division
NOAA/N/ORM2
U.S. Department of Commerce
Washington, DC 20235

National Oceanographic Data Center
NOAA/NESDIS E/OC21
2001 Wisconsin Ave., NW
Washington, DC 20235
202/634-7500

Northwest Region
National Marine Fisheries Service/NOAA
7600 Sand Point Way, NE
BIN C15700 - Bldg 1
Seattle, WA 98115-0070
206/526-6150

Southwest Region
National Marine Fisheries Service/NOAA
300 S. Perry Street
Terminal Island, CA 90931
213/514-6196

Alaska Region
National Marine Fisheries Service/NOAA
P.O. Box 21668
Juneau, AK 99802
907/586-7221

Northeast Region
National Marine Fisheries Service/NOAA
14 Elm Street, Federal Building
Gloucester, MA 01930
813/893-3141

Southeast Region
National Marine Fisheries Service/NOAA
9450 Koger Boulevard
St. Petersburg, FL 22703
813/893-3141

FWS

Region 1
U.S. Fish and Wildlife Service
Eastside Federal Complex
911 NE 11th Avenue
Portland, OR 97232-4181
503/231-2122

Region 2
U.S. Fish and Wildlife Service
500 Gold Avenue, SW
Albuquerque, NM 87103
505/766-2321

Table 3-1 (continued)

Region 4
U.S. Fish and Wildlife Service
Richard B. Russell Federal Building
75 Spring Street, SW, Room 1246
Atlanta, GA 30303
404/331-3594

Region 5
U.S. Fish and Wildlife Service
One Gateway Center, Suite 700
Newton Corner, MA 02158
617/965-5100

Region 7
U.S. Fish and Wildlife Service
1011 E. Tudor Road
Anchorage, AK 99503
970/786-3542

National Wetlands Inventory Program
U.S. Fish and Wildlife Service
18th and C Street, NW
Washington, DC 20240
202/235-2760

Division of Endangered Species
U.S. Fish and Wildlife Service
4401 North Fairfax Drive, Room 452
Arlington, VA 22203
703/358-2171

Office of Management Authority
U.S. Fish and Wildlife Service
Division of Endangered Species
4401 North Fairfax Drive, Room 432
Arlington, VA 22203
703/358-2093

MMS

Minerals Management Service
Branch of Environmental Studies (644)
Washington, DC 20240
202/343-7744

Minerals Management Service
Atlantic OCS Regional Office
1951 Kidwell Dr., Suite 601
Vienna, VA 22180
703/285-2165

Minerals Management Service
Gulf of Mexico OCS Regional Office
1201 Elmwood Park Boulevard
New Orleans, LA 70123
504/736-2896

Minerals Management Service
Pacific OCS Regional Office
1340 W. 6th Street
Los Angeles, CA 90017
213/894-7120

Minerals Management Service
949 E. 36th Avenue, Room 110
Anchorage, AK 99508-4302
907/261-4620

3.2.4 Presence of Other Discharges in the Area

The permitting authority must determine whether the presence of other discharges in the vicinity of the discharge of concern is likely to increase the potential for environmental impact and thus provide justification for additional monitoring requirements. This determination will depend on the number of other discharges in the area, their effluent flow, and the concentration of contaminants being discharged. Although individually the discharges in the area may not pose a significant threat to the marine environment, the cumulative effect of numerous discharges to the same receiving waterbody could result in a potentially significant impact on the marine environment in the vicinity of the discharges. Consideration of the presence of other discharges occurring in the vicinity of the discharge in question is particularly important in light of EPA's current emphasis on watershed management.

3.3 MONITORING REQUIREMENTS BASED ON PERCEIVED POTENTIAL ENVIRONMENTAL THREAT

Based on an evaluation of the criteria presented above, discharges can be placed into one of three categories based on their perceived potential for causing environmental impacts (Table 3-2):

- Minimal potential for causing environmental impacts,
- Moderate potential for causing environmental impacts, or
- High potential for causing environmental impacts.

The level and type of environmental monitoring required of a discharger will depend on its potential for causing environmental impacts. If sufficient information is not available to place a discharge into one of these general categories, a potential worst-case scenario should be assumed (i.e., the discharge poses a high potential for environmental impacts).

3.3.1 Minimal Potential Threat

Dischargers perceived to pose a minimal potential threat to the marine environment are those "minor" dischargers that do not discharge to stressed waters or sensitive biological areas and also do not discharge in the vicinity of other discharges where the combined effects of effluents could potentially cause unacceptable cumulative impacts. The permitting authority will have to determine the potential for cumulative impacts from multiple discharges on a case-by-case basis.

It is recommended that discharges perceived to be of minimal potential environmental concern be required to conduct effluent chemical characterizations, including whole-effluent toxicity testing, and to meet aquatic life and human health water quality criteria or standards. In addition, sediment contamination, which can involve deposition of toxicants over long periods of time, is responsible for water quality impacts in many

Table 3-2. Characterization of Section 403 Discharges Based on the Potential for Causing Environmental Impacts

Potential for Causing Environmental Impacts	Major/Minor Discharge	Location	Rationale
Minimal	Minor	- Does not occur in stressed waters; - Does not occur in the vicinity of sensitive habitats; AND[a] - Does not occur in the vicinity of other discharges where the potential for cumulative impacts exists.	- Excludes high-volume and/or highly toxic discharges. - Unreasonable degradation does not currently appear to be a problem. - Small likelihood of impacts to recreational, commercially, economically, or ecologically important species. - Small likelihood of contributing to cumulative impacts.
Moderate	Minor	- Occurs in stressed waters or in the vicinity of sensitive habitats OR[b] - Occurs in the vicinity of other discharges where the potenital for cumulative impacts exists.	- Excludes high-volume and/or highly toxic discharges. - In spite of low-volume and relatively nontoxic nature of the discharge, it may be contributing to currently stressed conditions. - May impact habitats of recreationally, commercially, economically, or ecologically important species that occur in the vicinity of the discharge. - May be contributing to cumulative impacts.
	Major	- Does not occur in stressed waters; - Does not occur in the vicinity of sensitive habitats; AND[a] - Does not occur in the vicinity of other discharges where the potential for cumulative impacts exists.	- May include high-volume and/or highly toxic discharges. - Unreasonable degradation does not appear to be occurring although because of the volume and nature of the discharge, the potential exists. - Depending on the volume, fate, and transport of the discharge and the toxicity and persistence of contaminants in the effluent, the potential exists for far-field impacts to recreationally, commercially, economically, or ecologically important species. - Small likelihood of contributing to cumulative impacts.
High	Major	- Occurs in stressed waters or in the vicinity of sensitive habitats OR[b] - Occurs in the vicinity of other discharges where the potential for cumulative impacts exists.	- May include high-volume and/or highly toxic discharges. - May be contributing to the unreasonable degradation (i.e., stressed conditions) of the environment. - May be contributing to cumulative impacts.

[a] All conditions must be true.
[b] Either one or the other condition must be true.

areas. Therefore, for discharges posing a minimal potential threat to the marine environment, it is recommended that near-field sediment contamination monitoring, in the form of sediment toxicity tests, be a requirement for 402 permits issued with 403 provisions or conditions.

3.3.2 Moderate Potential Threat

Both major and minor discharges can be considered to pose moderate potential threats to the marine environment. Minor discharges that are discharged to stressed waters or in the vicinity of sensitive biological areas or are discharged in the vicinity of other discharges where the combined effects of effluents could potentially cause unacceptable cumulative impacts should be considered to pose moderate threats to the marine environment. Major facilities that do not discharge to stressed waters or sensitive biological areas and that also do not discharge in the vicinity of other discharges where the potential for cumulative impacts exists should also be considered to pose moderate threats to the marine environment.

For discharges posing a moderate potential threat, field monitoring (in addition to sediment toxicity and effluent chemical characterization requirements for discharges presumed to cause minimal environmental impacts) should be a conditional part of 402 permits issued with section 403 provisions for "no irreparable harm." Such monitoring efforts should include physical transport studies as well as near-field measurements of contaminant concentrations in the water column, sediment, and benthic biota. Biosurveys and bioassessments should also be used to directly evaluate the overall biological integrity (structure and/or functional characteristics) of the aquatic community.

Current measurements and dye studies are recommended for tracking oceanic wastewater plumes. The purpose of such studies is to estimate the distance from the outfall that contaminated particles may travel before accumulating on the bottom and to evaluate the movement and spatial extent of the plume, the likelihood that the plume will reach the shoreline or other nearby areas, and the recirculation potential under short-term influences for existing discharges as well as the general circulation patterns in the vicinity of discharge sites. Seasonal current measurements and dye studies should be conducted to detect changes in current strength and direction (particularly wind-driven currents). The effects of a storm event on transport should also be investigated. Results of such studies can be used to identify areas potentially impacted by the discharge and to identify existing and potential problem areas for fish and sensitive life stages of marine organisms. Data from physical transport studies can also be used to calibrate and validate dispersion plume models.

Near-field monitoring of chemical concentrations can provide a spatial and temporal record of contamination in the water column. Water quality chemistry measurements should be made as part of a monitoring program to assess the effectiveness of permit

limitations in meeting water quality standards and to identify discharges that may not be in compliance with those standards. Water quality data can also be used to calibrate and verify mathematical models.

As discussed in the revised *Technical Support Document for Water Quality-based Toxics Control* (USEPA, 1991c), EPA encourages States to develop and adopt biological criteria in their water quality standards to fully protect aquatic habitats and provide more comprehensive assessments to determine the nonattainment of aquatic life uses. Biocriteria are numerical measures or narrative descriptions of the biological integrity of unimpaired natural systems. The biological communities in these waters become a reference and represent the best attainable conditions. The reference site then becomes the basis for developing biocriteria for major surface water types (in this case, coastal or marine waters). An assessment of biological integrity should include a measure of the structure and function of a community or species within a specific habitat. The specific definition of biological integrity selected by a State will form the basis for comparing impacted sites to an established reference condition.

For those discharges that pose a potential moderate threat to the marine environment, near-field biosurveys should be conducted and the results compared to reference conditions. Such biosurveys will provide a useful monitoring of both aggregate ecological impact and overall temporal trends in the condition of an aquatic system. Biosurveys can detect aquatic life impacts that other available assessment methods may miss, such as impacts caused by pollutants that are difficult to identify chemically or characterize toxicologically and impacts from multiple or unexpected exposures. Biological surveys should be used together with toxicity testing and chemical-specific analyses to assess the potential for nonattainment of designated aquatic life uses. Some acceptable protocols are currently available for developing biocriteria and conducting biosurveys for streams and rivers (Plafkin et al., 1989). EPA is planning to publish guidelines for developing biological criteria for wetlands and near coastal waters. These and other techniques developed by EPA's Office of Water and several States can be used as guidance to support biosurveys and bioassessments (Karr et al., 1986; Ohio EPA, 1987; Lenat, 1988; Schackleford, 1988; Maine DEP, 1987; Weber, 1973; USEPA, 1991a, b).

EPA has recommended in its *Technical Support Document for Water Quality-based Toxics Control* (USEPA, 1991c) that sediment criteria be adopted by States in their water quality standards. EPA has developed draft sediment criteria for five nonionic contaminants that are currently under Science Advisory Board review, and more criteria are being developed. For a discharge posing a moderate potential threat to the marine environment, it is recommended that near-field (i.e., within the zone of deposition) sediment chemical characterization (together with sediment toxicity testing) be a requirement for 402 permits issued with section 403 provisions for "no irreparable harm." Comparison of field measurements to sediment criteria could then be a reliable method for providing early warning of a potential problem. Such an early warning would offer an

opportunity to take corrective action before adverse impacts occur. For those numerous chemicals for which sediment criteria have not been developed, sediment chemistry monitoring can still be used to detect the presence of unsuspected "hot spots" and to assess the need for remedial action.

3.3.3 High Potential Threat

Discharges that may pose a high potential threat to the marine environment are "major" discharges that occur in stressed waters or near sensitive biological areas or that occur in the vicinity of other discharges where the potential for unacceptable cumulative impacts exists. It is recommended that, in addition to effluent and near-field monitoring, far-field monitoring studies be required for such discharges. The specific type of monitoring required will be dependent on site-specific circumstances.

Plume tracking studies should be conducted to estimate environmental concentrations of discharged contaminants and to determine potential exposure pathways and locations of field monitoring stations. Such studies may include measurements of effluent constituents in the receiving water, tracer studies, aerial surveys, measurement of sediment accumulation, or even indirect measures such as those provided by drogue or current-meter studies. These studies should be conducted on a seasonal basis to accurately assess changes that may occur in current strength and direction. In addition, a plume-tracking system using a new state-of-the-art technique, acoustical tracking, has been used in southeast Florida to indicate plume dynamics and to quantify mixing characteristics. The results of plume tracking should be used both directly and in combination with transport and fate modeling results to select far-field monitoring station locations. Such studies should also be used to assist in identifying the relative contribution of the discharge of interest to observed effects in near- and far-field environments.

Both near-field and far-field biosurveys, sediment chemistry monitoring, and sediment toxicity testing should be required. The location of monitoring stations should be based in part on the results of plume-tracking studies. When sensitive biological areas are present in the vicinity of the discharge, however, biological and sediment quality measurements should be conducted in these areas regardless of whether plume-tracking and modeling studies indicate a potential threat to these environments. The specific type of monitoring required will be dependent on the resources at risk as well as the chemical characteristics of the effluent. For stressed waters, the type of monitoring required will also be dependent on the type of stress observed (high levels of contaminants in fish tissues, hypoxic or anoxic conditions, coral bleaching, etc.).

3.4 SUMMARY

The preceding discussion has presented an overall approach for evaluating the level and general type of monitoring activities required as part of a permit issued to a discharger subject to 403 monitoring requirements. This approach relies on the best professional judgment of the permitting authority in making some of the determinations required in establishing monitoring requirements; for example, whether a discharge contributes to cumulative impacts resulting from multiple discharges. EPA will modify and expand upon the guidance presented here as improved tools for making these determinations are developed.

It should be noted that this guidance is designed to be applied as a tiered approach. That is, if initial monitoring results indicate that the potential environmental threat posed by a discharge may be greater than that originally suspected, then additional monitoring requirements may be required of the discharger. The discharger would be subject to the requirements of 40 CFR 122.62 or 122.63, whichever is applicable. For example, as a result of sediment toxicity tests, the monitoring requirements of a discharger originally thought to pose a minimal potential environmental threat may be increased to require monitoring activities defined for a discharger posing a moderate potential threat. As specified in 40 CFR 125.123(d)(4) of the Ocean Discharge Criteria, a permit may be modified (or revoked) at any time if the permitting authority determines that a discharge may cause unreasonable degradation of the marine environment. Therefore, the permitting authority can add monitoring requirements to a permit if the permitting authority deems it necessary to ensure that unreasonable degradation is not occurring, or will not occur, as a result of the discharge.

4. SUMMARY OF MONITORING METHODS

This chapter presents a review and evaluation of a number of monitoring and analytical methods that are available for use as part of CWA section 403 environmental assessments. Some of these methods are commonly used in monitoring and analytical programs, while others have not been widely used or may even be in the developmental/evaluation stage. The purpose of this effort is to provide EPA program managers and permit writers in the Regions and States with a basic understanding of methods available for use in 403 environmental assessments, as well as an understanding of the potential benefits and limitations of these methods. The information presented in this document is designed to assist program managers and permit writers in selecting monitoring and analytical program components appropriate for use in environmental assessment programs. The appropriateness of a particular method as part of a monitoring program will depend in part on the level of detailed information required, which will vary with the perceived threat of a discharge to the marine environment.

The most important component of a well-designed monitoring program is having competent, knowledgeable personnel conducting the collection and analysis of samples and data. This document is designed to provide only general guidance for environmental assessments, and not specific monitoring techniques or ecological information for each unique situation. This information can be provided only by technical personnel who are knowledgeable of monitoring and analytical techniques as applied to the discharge area they are charged with monitoring.

Numerous sources of information were accessed in the preparation of this methods evaluation. Sources contacted included EPA's Office of Research and Development, the 301(h) and National Estuary programs, the National Oceanic and Atmospheric Administration, the U.S. Fish and Wildlife Service, and university research laboratories.

The methods described in this document can be grouped into six categories: water quality, sediment quality, biological resources, human health risks, effluent characterization, and microcosms/mesocosms (Table 4-1). The relationship of each method to the section 403 ocean discharge guidelines is presented in Table 4-2.

Table 4-1. Sampling Method Categories

Water Quality

1. Physical Characteristics
2. Water Chemistry

Sediment Quality

3. Sediment Chemistry
4. Sediment Grain Size

Biological Resources

5. Benthic Community Structure
6. Fish and Shellfish Pathobiology
7. Fish Populations
8. Plankton
9. Habitat Identification Methods

Human Health Risks

10. Bioaccumulation
11. Pathogens

Effluent Characterization

12. Effluent Characterization

Mesocosms and Microcosms

13. Mesocosms and Microcosms

For each of the method sections, a brief description of the method is presented, followed by the rationale for use of the method described in terms of how the method addresses the section 403 ocean discharge guidelines, what information is generated, and how that information can be used in the 403 decision-making process. Each section describes the major monitoring design issues associated with each measurement type, as well as

Table 4-2. Matrix Illustrating Relationship of Method Types to the Section 403 Ocean Discharge Guidelines

Measurement Parameter	Ocean Discharge Guidelines									
	Quantities, composition, and potential bioaccumulation or persistence of the pollutants to be discharged	Potential transport of the pollutants by biological, physical, or chemical processes	Composition and vulnerability of potentially exposed biological communities	Importance of the receiving water area to the surrounding biological community	The existence of special aquatic sites	Potential direct or indirect impacts on human health	Existing or potential recreational and commercial fishing	Any applicable requirements of an approved Coastal Zone Management Plan (CZMP)	Such other factors relating to the effects of the discharge as may be appropriate	Marine water quality criteria
Physical Characteristics		✳								✳
Water Chemistry		✳				✳				✳
Sediment Chemistry	✳	✳				✳				
Sediment Grain Size	✳	✳								
Benthic Community Structure			✳	✳						
Fish and Shellfish Pathobiology			✳			✳			✳	
Fish Populations			✳	✳			✳			
Plankton			✳	✳			✳			
Habitat Identification Methods				✳	✳		✳	✳		
Bioaccumulation	✳	✳	✳			✳				
Pathogens						✳				
Effluent Characterization	✳					✳				✳
Mesocosms and Microcosms		✳	✳							

available laboratory and data analysis methods. The essential elements of appropriate, method-specific quality assurance and quality control programs and statistical design considerations are described, and an assessment of how the data generated can be used in a 403 assessment is provided. All of the information presented for each monitoring program component is summarized at the end of the section. Numerous references are also presented in Appendix A to provide the reader with additional sources of more detailed information concerning the use of the various methods.

This methods chapter is also intended to provide a summary of available information and to address the most important issues associated with the design and implementation of the monitoring program. Issues common to all monitoring methods include quality assurance/quality control (QA/QC) and statistical design.

Quality Assurance/Quality Control Considerations - A QA/QC plan should be developed to ensure the reliability and compatibility of data collected during the monitoring program. Quality assurance (QA), implemented at the management level, focuses on guidelines and procedures, such as delegation of authority and responsibility, to ensure data quality. Quality control (QC) centers on the technical activities, such as calibration or interlaboratory studies, needed to achieve specific data quality. QA is a discipline that begins with effective and conscientious work planning and ends with a carefully constructed set of checks and balances designed to ensure that uncertainties have been reduced to a known practical minimum (USEPA 1987c). QA is achieved by adopting specific guidelines and procedures to be followed during sample collection, sample analysis, data handling, and data analysis. These guidelines and procedures provide program quality control. QC includes the use of blanks and replicate samples to check field and laboratory sampling and handling techniques and to examine natural variability in contaminant concentrations.

Establishment of data quality standards is fundamental to the selection of monitoring methods and ensures comparability among data collected. A quality assurance plan should be developed by each monitoring program to standardize fundamental aspects of data collection and handling. Although the topics to be considered in a quality assurance plan will vary somewhat among programs, examples of some key considerations are provided in Table 4-3.

Data Quality Objectives - A major part of the QA effort is the establishment of Data Quality Objectives (DQOs). DQOs are specific, integrated statements and goals developed for each data or information collection activity to ensure that the data are of the required quantity and quality. DQOs should specify the desired sensitivity of sampling methods, timing of sampling, and numbers of samples to be collected. One function of establishing DQOs is to ensure that monitoring/data collection studies yield data adequate to meet their intended uses. DQOs (or data performance criteria) are also used to delineate QA/QC programs specifically geared to the data collection

Table 4-3. Key Topics to Be Addressed in Marine Monitoring Program Quality Assurance Plans

- Instructions for proper sample preservation and storage
- Chain of custody documentation
- Source of standard reagents
- Source or preparation of quality control samples
- Frequency of analysis of quality control samples
- Data Quality Objectives
- Analysis of QC samples
- Review of results
- Corrective action when unsatisfactory results are obtained in the analysis of QA/QC samples
- Calibration and maintenance of equipment

activities to be undertaken. Development of DQOs usually consists of three processes: (1) decision definition, (2) data use and needs identification, and (3) data collection program design. DQOs should be continually reviewed during data collection activities so that any needed corrective action may be planned and executed to minimize problems before they become significant.

DQOs play an important role in the selection of sample collection, sorting, and analysis methods. In fact, the selection of monitoring methods should be driven by DQOs. Any future modifications to monitoring protocols should be considered only after these new methods meet established data performance criteria. Data collected using different methods should not be compared unless information exists that supports such comparisons.

The methods sections that follow provide a discussion of existing monitoring methods. These methods provide a starting point for considering monitoring methodologies. New, cost-effective methods are sure to be developed for certain disciplines in the coming years. A key consideration when determining whether new methods are to be incorporated into the monitoring program is whether they meet the performance criteria of the QA/QC program. QA/QC considerations in the methods sections focus primarily on sample collection, processing, storage, and analysis.

QC Samples - Additional samples, either collected in the field or prepared from standard reference materials, are used to check for field or laboratory contamination, test laboratory accuracy, test field or laboratory reproducibility, and assess natural variability. Four basic types of QC samples should be submitted with each set of sediment or water quality samples (Table 4-4). Although the frequency with which these samples are collected will vary among programs, each should be collected at least once for every 20 samples or sampling event, whichever is more frequent.

Data from other existing monitoring programs will often be used in a monitoring program. Therefore, data will frequently be collected by a number of agencies following different sampling and analytical strategies. Under such conditions data comparability becomes a major QA/QC consideration. Data comparability must be evaluated based on both field and laboratory sources of variability. Individual programs will need to address this aspect of QA on a case-by-case basis.

Table 4-4. QC Sample Types

Travel (trip) Blank	Assess cross-contamination during handling and transport
Rinsate Blank	Assess contamination due to incomplete cleaning of sample equipment
Method Blank	Assess sample contamination due to reagents/sample handling in the laboratory
Field Split	Assess variability in sampling technique and/or laboratory handling

Statistical Design Considerations - Prior to the collection of data, how the data will be used to generate pertinent information essential to making sound decisions should be specified. It is further recommended that analytical performance criteria (e.g., minimally acceptable Type I and Type II error) be defined to establish quantitative expectations for the monitoring program. The link between data, performance, and decision-making should be specified a priori to ensure that appropriate data are obtained and that spatial and temporal variations are addressed by the monitoring plan.

Selection of the number of replicates is an important component of program design. The use of power analyses, examining alternative sampling and compositing strategies, will lead to an effective monitoring design strategy. These statistical techniques may mitigate the high costs of collecting and processing samples. As a general recommendation, samples of equal size should be collected whenever possible to simplify statistical analyses.

Composite Sampling - Composite sampling consists of mixing two or more replicates and/or samples. The chemical analysis of a composite sample provides an estimate of an average contaminant concentration for locations and/or times composing the composite sample (see space and time bulking below). Advantages of the composite sampling strategy are that it provides a cost-effective strategy when individual chemical analyses are expensive and it results in a more efficient estimate of the mean at specified sampling locations.

Because of the reduced sample variance, composite sampling may result in a considerable increase in statistical power. If the primary objective of a monitoring program is to determine differences in contaminant concentrations among sampling locations, composite sampling is an appropriate strategy.

Space-bulking consists of sampling from several locations and combining samples into one or more composite samples. Time-bulking involves taking multiple samples over time from a single location and compositing these samples. The use of space- and/or time-bulking strategies should be considered with the awareness that significant information concerning spatial and temporal heterogeneity may be lost.

Compositing of tissues provides a means to analyze bioaccumulation when the tissue mass of an individual is insufficient for the analytical protocol. If mixing species, tissue composites are likely to be composed of different proportions of species and different numbers, ages, and sexes of individuals. These differences among composites tend to confound the question of whether patterns of tissue residue concentrations are due to differences in locations or interspecific differences in bioaccumulation. Mixing of species is not recommended because of these confounding effects.

Composite sampling is not recommended if the objective of the monitoring program is to determine compliance with specified sediment contaminant concentration limits since this sampling method does not detect the true range of sediment contaminant concentrations in the environment. The adoption of composite strategies will depend on the objectives of individual monitoring programs.

Statistical Power - The large degree of temporal and spatial variability observed for many ecosystem state and rate variables requires collection of sufficient replicate samples to ensure an accurate description of the measure of interest. However, increases in replication increase sample processing costs. Power analyses assist in the allocation of sampling resources (stations, replication, and frequency) with regard to program finances and design (Sokal and Rohlf, 1981).

Power analyses may be applied to determine the appropriate number of sample replicates and/or subsamples in a replicate composite required to detect a specified difference (USEPA, 1987d). Statistical power increases with the increase of sample replicates. However, there is a point of diminishing return of statistical power with the addition of successive sample replicates (USEPA, 1987d). The number of replications required to detect a specified minimum difference is a function of the statistical power and the variance in the data. Power analyses require a priori knowledge of the variability in the data. A best guess or, preferably, variation observed in historical data is often used initially in the design of the monitoring program.

For composite samples, statistical power increases with an increase in the number of subsamples in each replicate composite sample (USEPA, 1987a); however, with the addition of successive subsamples to each composite, a diminished return of statistical power exists. For composites of greater than 10 replications, the increase of power is negligible given typical levels of data variability.

To improve the power of a statistical test while keeping the significant level constant, the sample size should be increased; however, because of constraints in cost and time, this option may not be available. Power analyses have shown that, for a fixed level of sampling effort, a monitoring program's power is generally increased by collecting more replicates at fewer locations. The number and distribution of sampling locations required to evaluate the effectiveness of the monitoring program will depend on the size and complexity of the site under investigation.

Power-Cost Analyses - The relative power of one design with respect to another is more meaningful when the relative costs of implementing alternative designs are taken into consideration. Power-cost analyses are fundamental in selecting appropriate sample/replicate number, sample location, and sampling frequency (Bros and Cowell, 1987; Cuff and Coleman, 1979; Ferraro et al., 1989; Millard and Lettenmaier, 1986).

A cost function that describes the cost per replicate sample is required. Power-cost formulations for parametric statistical analyses are of the form:

$$\text{Power-cost}_i = (1 - \beta_i) / (ci \times ni)$$

where **i** is a replicate sample collection and analysis scheme, **c** is the cost per replicate sample, **n** is the number of replicate samples, and β is the Type II error. Costs will depend on sample collection, sorting, analysis equipment, and protocols. Type II error (β) is a function of the number of replicate samples and the variance detected by a sampling and analysis scheme. The measured variance may be influenced by the sampling equipment used and the unit replicate sample size (e.g., as the unit replicate area approaches patch size, variance increases).

Since total costs are usually fixed, iterations of power-cost formulations for various sample processing scenarios (e.g., sample collection, sorting, and analysis schemes) and their comparisons will result in the selection of the appropriate monitoring methodology. Sokal and Rohlf (1981) provide a series of formulas for calculating the most efficient sampling design for a given cost.

4.1 PHYSICAL CHARACTERISTICS

Physical characteristics are typically measured in situ and include temperature, salinity, density, depth, turbidity, and current velocity and direction. Physical data are used to characterize the water column, verify hydrodynamic models, and indicate spatial and temporal variations.

4.1.1 Rationale

Of the 10 guidelines used to determine unreasonable degradation or irreparable harm, the following can be assessed by evaluating physical characteristics:

- Potential transport of the pollutants by biological, physical, or chemical processes and
- Marine water quality criteria.

Most chemical and biological processes in the marine environment are affected, either directly or indirectly, by physical characteristics of the marine environment (Thomann and Mueller, 1987). Consequently, physical characteristics are used to determine plume and sediment movement (hydrodynamics and fate and transport modeling) and to provide ancillary information to interpret other water chemistry variables. In addition, to ensure protection of the characteristic indigenous marine community, national temperature criteria have been established in the "Gold Book" (USEPA, 1986d).

All biological monitoring should include consistent physical habitat variable measurements and physical habitat matches between sampling sites and reference sites or identified conditions in order to accurately assess the condition of biological resources in the area of sampling. Physical measurement data such as grain size, depth, salinity, temperature, dissolved oxygen, and vegetative cover should be measured along with biological variable measurements.

4.1.2 Monitoring Design Considerations

The main uses of physical data are to characterize the area in the vicinity of the discharge site and to serve as ancillary information for other variables. As a result, temperature, salinity, density, turbidity, and depth (depth of sample and depth to bottom) should be collected at every station location. The cost of the various methods is controlled by the level of automation and, in comparison, physical data collection is relatively inexpensive. In many instances, the sampling location and frequency will be controlled by other more expensively collected variables. The number and location of current measurements should be based on the complexity of the circulation patterns and effluent characteristics.

If a mathematical model will be used to assess the effects of ambient physical characteristics and the fate and transport of the discharged effluent, the monitoring program should be designed to provide the model with the required forcing boundary condition data. It may also be necessary to collect meteorological data (e.g., wind speed and direction, temperature, pressure) for modeling efforts since plumes can be affected by wind effects.

General recommendations for sampling locations include the following:

- To predict potential transport of the pollutants by biological, physical, or chemical processes, sampling stations need to be located in the vicinity of the zone of initial dilution (ZID) boundary, at control sites, and in impacted areas to allow adequate correlations to be made between water quality, oceanographic measurements, toxic substances, and biological data (USEPA, 1982a). Other locations may include the shoreline in swimming and shellfishery areas and within the ZID (Koh, 1988).

- Additional monitoring stations should be located near other pollutant sources, as applicable, to allow the effects of the subject discharge to be distinguished from these sources.

- Several samples should be taken at various depths across the waste field as the plume crosses the ZID boundary.

- Sampling depths are generally acceptable if taken at standard depths (Pond and Pickard, 1983); however, features of water masses observed in profiling for salinity, temperature, and turbidity should take precedence in establishing sample depths. When using in situ methods (e.g., CTD), temperature and salinity measurements should be taken at 1-meter intervals over the entire depth profile (to within 1 meter of the surface and bottom). In areas of high stratification, a smaller interval would be appropriate.

- As a practical minimum when using bottle sampling techniques, samples for temperature, salinity, and turbidity should be taken at four depths in the vertical profile: (1) 1 meter below the surface, (2) 1 meter above the bottom, (3) 1 meter above the pycnocline, and (4) 1 meter below the pycnocline. If the waters are too shallow or no stratification occurs, it would be appropriate to take the latter two samples at evenly spaced distances between the top and bottom samples. In many cases, these depths will coincide with variables described in other sections.

General recommendations for sampling frequency include the following:

- Sampling frequency should be high enough to differentiate between high-frequency natural events (e.g., tides, storms) and mean conditions.

- To establish meaningful estimates of the plume behavior, it is essential that the monitoring program encompass the range of conditions that might occur. In particular, to obtain seasonal variations, measurements should extend for at least 2 years.
- Sampling frequency of temperature and salinity should be selected to provide data for critical periods (e.g., time of seasonal maximum and minimum stratification, low net circulation, time and duration of coastal upwelling, and exceptional biological activity).
- In monitoring the other physical characteristics of the receiving water, sampling events and frequencies should be timed to provide information during critical environmental periods.
- More frequent sampling in fish and shellfish harvesting areas, and in other biologically sensitive areas, may be required.

Moored instrument arrays provide a cost-effective method of continual sampling over long periods of time (from days to months) for many physical characteristics. More commonly used at remote offshore locations, subsurface arrays can provide unattended long-term data collection in estuarine environments for both physical and chemical parameters. Water depth, velocity (speed and direction), temperature, and conductivity or salinity are the most commonly measured characteristics. Estimates of total suspended solids and chlorophyll can be recorded also, using turbidometers, transmissometers, or fluorometers. Dissolved oxygen concentrations can also be measured. Sensors capable of in situ measurements of nutrient and trace contaminant concentrations are under development.

Moored arrays have the potential to provide synoptic coverage at predetermined intervals, which can range from minutes to days. The longer the interval between data recordings, the longer the array can function without replacing the power supply (usually batteries) or the recording medium (cassette tape or computer memory chips). Real-time telemetry of data to a shore station is possible with the more sophisticated (and more expensive) instrumentation packages. More commonly, the instrument array is retrieved at regular intervals and the collected data post-processed. Moored arrays that are deployed for long periods of time are susceptible to many types of interference.

4.1.3 Analytical Methods Considerations

Methods available for measuring physical characteristics include instruments that range from simple mercury-filled thermometers that provide one observation of temperature to state-of-the-art CTD (conductivity-temperature-depth) meters that provide vertical profiles of salinity and temperature. Methods and/or equipment discussed in this section are listed in Table 4-5.

Table 4-5. List of Methods and Equipment

Temperature

- mercury-filled or dial-type centigrade thermometer
- thermistor
- reverse thermometer
- bathythermograph
- CTD

Salinity

- Knudsen titration
- conductivity cells (CTD instruments)

Density

- direct (hydrometers, pycnometers, magnetic float densimeters)
- indirect (function of salinity, temperature, and pressure)

Depth

- fathometer
- meter wheel
- hydrostatic pressure
  - bourdon tube
  - strain gauge

Turbidity

- light transmissometer
- Secchi disc
- nephelometer
- turbidimeter

Current Measurements

- Eulerian Method (speed and direction)
  - mechanical
  - electromagnetic
  - acoustic
- Lagrangian Method
  - drogues
  - surface drifters
  - seabed drifters

Dye Studies

- fluorometer

Temperature

Temperature measurements may be made with any good grade of mercury-filled or dial-type centigrade thermometer, or a thermistor (USEPA, 1983a, Standard Method 170.1). Surface temperatures (from bucket samples) may be taken using an ordinary mercury thermometer, taking care not to expose the bucket to the sun (heating) or to the evaporating influence of the wind (cooling).

For measuring subsurface temperatures, the basic instrument is the "protected reversing thermometer," a mercury-in-glass thermometer that is attached to the water sampling bottle. When the sample bottle is closed, the thermometer is inverted. The mercury "breaks" at a particular point and runs to the other end of the capillary, thereby recording the temperature of the water at the depth where the sample was taken (UNESCO, 1988).

A bathythermograph (BT) consists of a thermistor and an electronic pressure transducer. Wire extending from the BT to the vessel relays measures of temperature and pressure. BTs may be operated from stationary or slow-moving (<4 knots) vessels. Expendable bathythermographs (XBTs) are based on tracking an expendable, free-falling temperature sensor. The system is designed to operate from a stationary or moving vessel (<15 to 20 knots) typically in deep waters. Wires extending from the free-falling transducer to the vessel relay temperature and depth recordings. The wires are cut when the probe reaches its maximum depth. BTs and XBTs do not record measures of salinity. If it has been determined that density is not related to salinity, bathythermographs may be a cost-effective tool. Monitoring of temperature and pressure using XBTs is higher in cost since the probe is lost after each vertical profile.

Conductivity-temperature-depth meters (CTDs) are the tool of choice since density measures are typically dependent on salinity, temperature, and depth (see Density below). Measures of density are important in identifying and tracking the movements of waterbodies and in determining the depth at which a water mass will settle at equilibrium. CTDs coupled with a computer can continuously integrate temperature and conductivity measurements with depth. The CTD/computer combination provides real-time readouts that allow immediate modifications in CTD position and ensures comprehensive water column coverage during the survey.

Salinity

The Knudsen titration method determines chlorinity by titration with a standard silver nitrate solution, and salinity is then computed from a standard formula (Pond and Pickard, 1983). This titration method is practical but not convenient to use on board a ship (APHA, 1989).

Conductive cells are used for in situ measurements, and a variety of such instruments are now available from several manufacturers (Pond and Pickard, 1983). A CTD unit consisting of conductivity, temperature, and depth sensors is lowered through the water on the end of an electrical conductor cable, which transmits the information to recording units on board ship (UNESCO, 1988).

Density

Direct measurement of density is generally not accurate enough for most oceanographic considerations (Pond and Pickard, 1983). Nevertheless, devices such as hydrometers, pycnometers, and magnetic float densimeters are sometimes used when great accuracy is not required.

In the absence of adequate means to measure seawater density accurately and quickly, it is common practice to determine density indirectly from salinity, temperature, and pressure. The density (or the specific gravity) of seawater depends on the temperature, salinity, and (as a result of the slight compressibility of water) pressure. In practice, temperature, salinity, and pressure are measured and the density is read from tables that have been prepared from laboratory determinations (e.g., Eugene, 1966). Algorithms for automated data processing have been developed by UNESCO and are applicable for salinities as low as 2 ppt (Fofonoff and Millard, 1983). For lower salinities, see Hill et al. (1986).

Depth

The simplest method to determine depth is to pass the wire that is attached to the instrument over a meter wheel with a known circumference and a counter. In calm conditions and negligible currents, the length of wire used will be the actual depth.

Depth may also be estimated using a bourdon tube. The tube moves either the slider of an electrical potentiometer or a strain-gauge pressure transducer, and the resulting current corresponds to the depth of the instrument.

The total water depth at a particular station is typically determined with acoustic transponders, which are widely available, are relatively inexpensive, and are accurate to within 0.3 to 0.7 foot (Clausner et al., 1986).

Turbidity

To determine the transmission of visible light through the water, the simplest device is the Secchi disc, a black-and-white plate about 30 cm in diameter. The Secchi disc is lowered into the sea, and the depth at which it is lost to sight is recorded (Pond and Pickard, 1983). Measurements of Secchi disc depth are probably the most widely used means of estimating light penetration, and they can be used to estimate the attenuation

coefficients for collimated and diffuse light. As a result, the depth of the euphotic zone may be estimated. Secchi disc readings vary with the observer because of interpersonal differences in visual ability; therefore, caution must be exercised when comparing Secchi disc readings taken by a variety of observers.

Turbidity is the measure of particulate matter (plankton and suspended sediments) in the water column. Measurements, either in situ or remote, are made by spectrophotometric methods. This involves passing a light of known intensity and wavelength through a water sample of precise length. The amount of light received by a receptor is the percentage of light that has not been absorbed or reflected within the path length. In remote measurements, water samples are obtained from discrete depths and then analyzed on deck. This method of sampling is quick and accurate, but it is labor-intensive and is restricted to sampling only at those depths where water samples were taken. In situ sampling involves passing a light transmissometer (usually as part of a CTD system) through the water column. This provides for much higher sampling rates and less labor. To correlate data taken from different instruments, all values should be expressed as a percent transmittance for a standard path length.

Current Measurements

The two basic ways to describe fluid flow are the Eulerian method, in which the velocity and direction are stated at every point in the fluid, and the Lagrangian method, in which the path followed by each fluid particle is stated as a function of time.

Eulerian Method

Mechanical meters include Savonious rotors, ducted impellers, drag inclinometers, and propeller-type meters. Savonious rotor current meters use a unidirectional rotor on a vertical axis of rotation and a vane that senses the horizontal direction of flow. Although these meters have been used for many years in oceanographic work, they are not suited for operation in shallow-water environments, which are exposed to wave and swell activity. Ducted impeller current meters have been designed to reduce the problems associated with current measurements in the presence of waves (Brainard and Lukens, 1975). Davis-Wellar propeller-type meters are also designed to operate in the presence of waves.

Electromagnetic current meters measure the instantaneous horizontal and vertical velocity components at a flow sensor that contains a wire coil and two orthogonal pairs of electrodes. The coil produces a magnetic field, and the electrodes measure the voltage gradient across the coil, which is induced by the water as it flows through the field. The characteristics of these meters make them suitable for use in the presence of waves and shallow water (McCullough, 1977). The most recent electromagnetic current meters are equipped with several probes to measure other physical characteristics of the receiving water as well.

Acoustic current meters determine the current velocity by emitting a short acoustic pulse and measuring the Doppler frequency shift of the backscattered signal. Small particles in the water column (i.e., plankton and suspended sediments) that float passively with the current act as reflectors of the acoustic signal. If the particles are moving toward the sound source, the frequency is shifted higher, whereas it is shifted lower if the particles are moving away. The frequency shift is proportional to the relative velocity between the source and the reflectors. By range gating, return signal velocity measurements at discrete depths can be made. This lets one instrument take remote samples throughout the water column from a depth of 3 meters to over 1,000 meters. Another advantage of this technology is that the current meter can be placed on a ship and sampling can be conducted while the ship is under way. This allows for a synoptic measurement of the current field over a large area with the use of only one instrument. Acoustic current meters are suitable for use in the presence of waves, but the accuracy of the measurements decreases close to the sea surface. These instruments are also expensive and require highly trained technicians to maintain them.

Another technology employs the use of land-based radar systems that send out electromagnetic waves and measure the reflected energy from the surface waves (Appell and Curtin, 1990; Appell and Woodward, 1986). Since this system is set up on the shore, it works out to only a few dozen kilometers offshore and measures only the current velocity in the top meter of the water column. This system does have the advantage that a ship is not needed to deploy or retrieve the instrument.

Lagrangian Method

Most drogues are drogue-buoy systems consisting of a small marker buoy that is tracked at the surface and a larger submerged drogue portion that is set at the desired depth by a connecting line between the two portions (USEPA, 1982a). The drogue portion must be weighted and ballasted so that the drogue assembly has sufficient negative buoyancy to keep both the drogue and connecting line in their intended vertical orientation and to keep the buoy mast upright. Drogues are intended to passively drift with the currents at a specified depth. In reality, some error is introduced in the drogue trajectories by wind drag on the exposed portion of the marker buoy; by the relative surface current drag on the submerged portion of the surface buoy; and, for deep drogues with long lines, by the relative current drag on the connecting line. If possible, it is best to avoid drogue studies under high-wind conditions, especially when measuring lower current speeds in deeper waters. Drogues can be used for periods ranging from a couple of days to many months and can be tracked by satellite.

Most drogues can be classified into one of the following four categories: (1) parachute drogues, (2) cruciform drogues, (3) window shade drogues, and (4) cylindrical drogues. Of these types, parachute and cruciform drogues are the most widely used (USEPA, 1982a).

Surface drifters are used to measure the average path of currents at the surface. Drift bottles and drift cards are the types most commonly used. Vertical drift bottles and drift cards are useful for evaluating the movement of effluent that reaches the surface. Horizontal drift cards, which float on the surface, may be used to determine the potential movement of surface slicks due to oils or other floatables that form a surface film. Because these movements are influenced largely by the wind, horizontal drift cards should be released under several different conditions (Grace, 1978).

Seabed drifters measure the average path of currents near the sea floor. They are useful for determining the fate of waste materials subject to transport by bottom currents. This includes settleable solids and any portion of the effluent that remains near the bottom. The drifters provide information on the net movement of a waste field along the bottom, including where the waste field may reach the shoreline, and a rough estimate of how long it may take. If a sufficient number of drifters are recovered, they may indicate areas of possible shoreline contamination (Grace, 1978).

Dye Studies

Dye studies involve the continuous or plug injection of a tracer (rhodamine WT or rhodamine B) into a waste stream before it is discharged. Fluorometers are used to estimate the concentration of the sample containing a fluorescent substance. The intensity of the fluorescent light is compared with readings for samples with known concentrations (standards) under the same conditions (Wilson, 1986). The advantages of dye tracing are (1) low detection and measurement limits and (2) simplicity and accuracy in measuring dye tracer concentration techniques.

Acoustic technology is currently being tested to characterize and track oceanic wastewater plumes. Results from several tests conducted off the coast of Florida and dye release experiments have been published (Demmann et al., in press).

4.1.4 QA/QC Considerations

All environmental monitoring programs should have a written and approved quality assurance project plan (USEPA, 1987c). Tables 4-6 and 4-7 list sample preservation and storage requirements and recommended analytical methods for salinity and temperature. When several methods are available, the selection should be made by comparing the accuracy and precision of the candidate methods for the parameter range expected at the site. All monitoring programs should follow manufacturers' recommended calibration procedures. In addition to these general considerations, the following specific recommendations should be adopted as appropriate:

- Each temperature-measuring instrument should be calibrated against a precision thermometer, certified by the National Bureau of Standards, at least every week (USEPA, 1987c). It is recommended that calibration be calculated daily when temperature violation is suspected. Temperature probes may, at

Table 4-6. Recommended Sample Preservation and Storage Requirements

Parameter	Volume Required (mL)	Container	Preservative	Holding Time
Salinity	240	G (with paraffined corks)	None	1 hour (longer if properly sealed airtight)
Temperature	1,000	P,G	None	Immediate

Note: P = polyethylene, G = glass

SOURCES: APHA, 1989; USEPA, 1983.

Table 4-7. Recommended Analytical Methods

Parameter	Method	Precision	Detection Limit Desired	Significant Figures Desired
Salinity	Induction salinometer or titration. Method 2520 (APHA, 1989).	titration: ±0.05 ppt	1 ppt	4
Temperature	Bathythermograph or thermometric. EPA Method 170.1 (USEPA, 1983a).	±0.05°C	—	3

Note: The water column, being a 3-D medium, requires greater numbers of samples to be collected (versus a 2-D medium) in order to be adequately described.

best, be accurate to within one-tenth of a degree Celsius. Temperature probe systems are rarely linear over large temperature ranges and must be checked against research-grade laboratory thermometers (APHA, 1989).

- Salinity probe systems offer moderate accuracy but should be cross-checked by discrete water samples analyzed by induction-type laboratory salinometers (APHA, 1989). Two standard determinations should be made before the start of each series of samples. In addition, one standard sample should be analyzed to monitor instrument drift. It is recommended that duplicate determinations be made for at least 10 percent of the samples analyzed.

- Many multiprobe in situ measurement systems incorporate depth measurements by use of pressure transducers. The accuracy and precision of such systems must be periodically checked. A pressure calibration can be done in a laboratory pressure chamber. Daily calibration may be done in calm waters of a known depth (e.g., inside a lagoon or a marina). Precision is determined by multiple measurements at the same depth. Accuracy is evaluated by comparison to measurements made with a heavily weighted line.

- Instruments that measure turbidity require regular calibration. In situ instruments are most accurately calibrated by the use of primary standards, usually a carefully prepared suspension of Formalin or other approved standard. Transmissometer-type instruments can be calibrated by comparison to solutions of known total suspended solids. These solutions should approximate the turbidity of the water that is to be sampled. For other instruments, including those nephelometers or turbidity meters that measure discrete samples, either on deck or in the laboratory, calibration using an approved primary standard suspension is recommended (APHA, 1989). Calibration in all cases should be conducted at the start of each series of analyses and after each group of 10 successive samples. Duplicate analyses should be conducted on at least 10 percent of the total number of samples (U.S. EPA, 1987c).

- Transmissometers can be calibrated in air and must be strictly cleaned.

- A standard suspension of Formalin, prepared under closely defined conditions, is used to calibrate the nephelometer (USEPA, 1983a, Method 180.1). The nephelometer should be calibrated at the start of each series of analyses and after each group of 10 successive samples. Duplicate analyses should be conducted on at least 10 percent of the total number of samples (USEPA, 1987c).

- Current meters should be calibrated before and after each major deployment of instruments.

- UNESCO (1988) presents details on the calibration of CTD instruments.

- Fluorometer calibration performed prior to use with a series of prepared concentrations, blanks, and spikes is recommended as a quality control check (Wilson, 1986).

4.1.5 Statistical Design Considerations

Temporal Analyses

In general, one would expect to find temporal and spatial patterns in physical characteristics. As a result, the primary purpose of measuring physical characteristics is to describe what these patterns are (e.g., range, seasonal variations) and to determine the primary physical mechanisms affecting the monitored area. As a result, statistical comparisons between sampling locations and sampling periods are not common except for turbidity. In this instance, the reader is referred to Section 4.2, Water Chemistry, for analysis approaches that would be appropriate. All raw data should have error limits associated with them to indicate the level of confidence in the data.

Tidal and seasonal variability in the distribution and magnitude of water column physical characteristics is typically observed (Day et al., 1989). Time series analyses (e.g., temporal autocorrelation and spectral analyses) may be necessary to examine the effects of the cyclical influences and/or filter out these cyclic forcing functions in order to examine long-term temporal trends.

Graphical Representation

The physical characteristics data described in this section are typically summarized in graphical form. The most common summary graphs include temperature-depth profiles, salinity-depth profiles, and temperature-salinity plots. Physical oceanographers typically analyze characteristic diagrams of water properties. The most common curve is a temperature-salinity (T-S) plot.

Alternatively, horizontal sections (e.g., contour maps) of properties are useful for displaying geographical distributions. Multiple physical characteristics may be displayed by displacing one graph above the other. More details about analyzing characteristic diagrams can be found in Pickard and Emery (1982).

Current data can be displayed in numerous fashions including stick plots, a time series of rectangular components, and horizontal maps (Pickard and Emery, 1982). These figures are typically plotted after periodic components have been removed through spectral analysis, leaving only the residual nonperiodic components.

4.1.6 Use of Data

Data describing the physical parameters can be used to determine water column stability, characterize oceanographic conditions in the discharge area, determine initial dilution, assist in the prediction of plume behavior, identify the area impacted by the discharge, and identify existing and potential problem areas for fish and sensitive life stages of marine organisms (e.g., upwelling areas).

Salinity and temperature can be used, together with current speed and direction, to aid in describing mass movement of diluted waste plumes to far-field sites where impacts must be assessed (Wright, 1988). Furthermore, salinity, temperature, and currents are used to assist in evaluating the results of benthic and other biological responses.

Current-speed data may be used to determine the distance from the outfall that the sediment will travel before accumulating on the bottom.

Dye studies are particularly useful to evaluate the movement of the plume, the likelihood that the plume will reach the shoreline or nearby biologically sensitive areas, the potential for re-entrainment of previously discharged effluent, and the recirculation potential under short-term influences for existing discharges, as well as the general circulation patterns in the vicinity of discharge sites (USEPA, 1982a).

Physical characteristics data are used to calibrate and validate hydrodynamic and dispersion plume models (Davis, 1988; Leighton et al., 1988; USEPA, 1982a). For relocated or proposed discharges, numerical circulation and transport models are the most useful methods for assessing the effects of ambient currents and stratification on dispersion and transport of the waste field and for estimating the potential for recirculation of previously discharged effluent. Statistical analysis can be performed to forecast plume direction under varying physical conditions.

Turbidity data can be used to estimate the reduction in light transmittance. Reduction of the depth to which sunlight penetrates, due to an increase in turbidity, will cause a decrease in the compensation depth of phytoplankton, resulting in reduced biological growth (Thomann and Mueller, 1987).

4.1.7 Summary and Recommendations

<u>Rationale</u>

- Most chemical and biological processes in the marine environment are affected, either directly or indirectly, by the physical characteristics of the marine environment.

- Physical data can be used to determine plume and sediment movement (hydrodynamics and fate and transport modeling) and to provide ancillary information to interpret other water chemistry variables.
- National temperature criteria to ensure protection of the characteristic indigenous marine community have been established in the "Gold Book" for temperature (USEPA, 1986d).

Monitoring Design Considerations

- In general, data on temperature, salinity, density, turbidity, and depth (depth of sample and depth to bottom) should be collected at every station location. Sufficient data should be collected in the vertical profile for temperature and salinity. Common practice is to collect one data point per meter of depth.
- The number and location of current measurements should be based on the complexity of the circulation patterns and the effluent characteristics. Stations should be located in the vicinity of the zone of initial dilution (ZID) boundary, at control sites, and in impacted areas.
- If a mathematical model will be used to assess the effects of ambient physical characteristics and the fate and transport of the discharged effluent, the monitoring program should be designed to provide the model with the required forcing boundary data.
- Data should be collected at a frequency sufficient to characterize seasonal patterns and critical periods (e.g., time of seasonal maximum and minimum stratification, low net circulation, time and duration of coastal upwelling, and exceptional biological activity).
- Estimates of the error should be made with each variable.

Analytical Methods Considerations

- Temperature measurements may be made with:
  - Mercury-filled or dial-type centigrade thermometer
  - Thermistor
  - Reverse thermometer
  - Bathythermograph
  - CTD instrumentation
- Salinity measurements are preferably made with:
  - Conductivity cells (CTD instruments)

- Density measurements are preferably made with:
  - Indirect methods (function of salinity, temperature, and pressure)
- Depth measurements are preferably made with:
  - Hydrostatic pressure techniques
- Turbidity measurements may be made with:
  - Light transmissometer
  - Secchi disc
- Current measurements may be made with:
  - Eulerian methods - propeller-type meters, electromagnetic meters, acoustic-doppler meters, etc.
  - Lagrangian methods - drogues, surface drifters, etc.
- Plume studies may be performed with:
  - Fluorescent dyes injected into the waste stream
  - Emerging acoustic technologies

QA/QC Considerations

- A multistep QA/QC program must be implemented to verify that all calibrations were conducted and properly applied to data and, where possible, to ensure historical verification.
- All equipment must be calibrated regularly.
- Blank, spike recovery, and replicate analyses are recommended quality control checks for those parameters to which they can be applied.
- For replicates, a minimum of three repetitions is required for statistical significance.

Statistical Design Considerations

- The primary purpose is to describe the patterns of physical characteristics (e.g., range, seasonal variations) and to determine the primary physical mechanisms affecting the monitored area. As a result, statistical comparisons between sampling locations and sampling periods are not common except for turbidity.
- Common graphical summaries include:

- Temperature-depth profiles, salinity-depth profiles, and temperature-salinity plots.
- Horizontal sections (i.e., contour maps) of properties.
- Time series data, which are often displayed as a function of time with multiple physical characteristics displayed by placing one graph above the other.
- Current data, which can be displayed with stick plots, a time series of rectangular components, and horizontal maps.

Use of Data

- Physical characteristics are used to determine water column stability, to characterize oceanographic conditions in the discharge area, to determine initial dilution, to assist in the prediction of plume behavior, to identify the area impacted by the discharge, and to identify existing and potential problem areas for fish and sensitive life stages of marine organisms (e.g., upwelling areas).
- Statistical studies can be performed to forecast plume direction under varying physical conditions.
- Dye studies are particularly useful to evaluate the movement of the plume, the likelihood that the plume will reach the shoreline or nearby biologically sensitive areas, the potential for re-entrainment of previously discharged effluent, and the recirculation potential under short-term influences for existing discharges, as well as the general circulation patterns in the vicinity of discharge sites.
- Physical characteristics data are used to calibrate and validate hydrodynamic and dispersion plume models.
- Turbidity can be used to estimate the reduction of light transmittance. Reduction of the depth to which sunlight penetrates, due to an increase in turbidity, can reduce biological community growth.

4.2 WATER CHEMISTRY

Monitoring of water chemistry parameters is conducted to evaluate the quality of receiving waters. Commonly employed EPA methods were intended for use in the detection of pollutants at high concentrations relative to those usually found in natural waters. Much attention has been devoted recently to the need for the development of more appropriate methods, but evaluation and validation of these methods have not been conducted on a wide scale. Until these and other QA measures are developed, the traditional EPA methods are generally the methods of choice for the analysis of water quality. *Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound* (USEPA, 1986-1991) contains discussions of QA/QC concerns, sampling considerations, and recommended instrumental methods. The *Compendium of Methods for Marine and Estuarine Environmental Studies* (USEPA, 1990b) includes a number of method variations for nitrogen species, phosphorus, and chlorophyll.

4.2.1 Rationale

Of the 10 guidelines used to determine unreasonable degradation or irreparable harm, the following can be assessed by evaluating the water chemistry:

- Potential transport of the pollutants by biological, physical, or chemical processes;
- Potential direct or indirect impacts on human health; and
- Marine water quality criteria.

The presence of toxic material in marine waters due to point source discharges can have adverse ecological and human health effects. Near-field monitoring of chemical concentrations can provide a spatial and temporal record of contamination in the water column. Water quality chemistry measurements should be made as part of a monitoring program to assess the effectiveness of the permit guidelines in preventing unreasonable degradation of or irreparable harm to the marine environment.

4.2.2 Monitoring Design Considerations

Selection of Analytes

The chemicals that should be included in the monitoring program are those chemicals known or suspected to be in the discharge, as well as possible by-products. Even though potentially toxic compounds in the discharge may not be fully known, the objectives of the program must be clearly defined. It may be necessary at the beginning of the program to conduct an analysis for all chemicals on the EPA-defined Priority Pollutant, Hazardous Substance, or Target Compound/Analyte Lists to determine which are present; however, detection in the environment does not always correlate with

biological risks and effects. Furthermore, many of the chemicals that pose risks to marine systems are hydrophobic. Water column monitoring for hydrophobic chemicals may indicate concentrations that are several orders of magnitude below concentrations in sediments and biota. Unless compelling site-specific conditions warrant otherwise, water column monitoring for hydrophobic chemicals is not recommended. Limitations in analytical methodology, modeling techniques, and toxicological data restrict the usefulness of the resulting data. In some cases, levels of contaminants that are of concern to the health of organisms are below the lowest limit of detection of the best available analytical methodology.

Sampling Considerations

Selection of sampling locations and choice of sampling techniques are crucial decisions to be made before the sampling effort is initiated. *Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound* (USEPA, 1986-1991) contains discussions of quality assurance requirements and sampling considerations that are applicable to water quality monitoring. Sampling must incorporate sites within the zone of initial dilution, extending out to areas of low contaminant concentration and farther to unaffected areas to address the complete spatial variability.

Water column sampling design must consider not only the horizontal location of the sampling stations, but also the vertical location within the water column and the time of sample collection. Because the majority of chemical analyses are performed, by necessity, on discrete samples, consideration should be given to the number of depths at which samples are collected. Obviously, such decisions will be based on site-specific characteristics, such as water depth, contaminants of concern, suspected sources, and the sensitivity or importance of local biota. Costs related to the laboratory analyses should also be weighed against the number of samples desired.

Water Column Sampling Equipment

Water column samples are frequently collected using bottle samplers. These are simple devices, usually consisting of cylindrical tubes with stoppers at each end and a closing device that is activated by an electrical signal. Each bottle samples a discrete parcel of water at a designated depth. Multiple samplers are fixed on a rosette frame in order that several depths may be sampled during one cast and/or that replicate samples may be collected at a particular depth. The most commonly used bottle samplers include the Kemmerer, Van Dorn, Niskin, and Nansen samplers (USEPA, 1987c). Alternatively, a pump may be used to sample the water column (USEPA, 1987c).

Depending on the depth of the water at the sampling site, samples should be taken at a minimum of four depths in the vertical profile: (1) 1 meter below the surface, (2) 1 meter above the bottom, (3) 1 meter above the pycnocline, and (4) 1 meter below the

pycnocline. If the waters are too shallow or no stratification occurs, it would be appropriate to take the latter two samples at evenly spaced distances between the top and bottom samples.

Caged Organisms

The California Mussel Watch Program and the National Oceanic and Atmospheric Administration (NOAA) Status and Trends Program have employed the use of caged transplanted mussels to monitor bioaccumulation of toxic chemicals over space and time (Ladd et al., 1984; Goldberg et al., 1978). Caged sentinel species offer several advantages:

- They concentrate contaminants (up to 10^2-10^5 fold vs. ambient waters), facilitating laboratory analyses of contaminant concentrations.
- They provide a means of temporally integrating water quality conditions.
- They provide an assessment of biological availability of water column contaminants.
- They may be deployed and maintained in a number of diverse locales.

However, the disadvantages of caged organisms include the cost of transplanting organisms, the possibility of loss of the cage/buoy system, and the possibility of introducing a "nuisance" species. Species have different bioaccumulation potentials for various contaminants. It is recommended that multiple sentinel species be deployed to ensure that a number of contaminants are sufficiently evaluated and a comprehensive characterization of water contaminants is conducted. Unfortunately, formulations that would allow comparisons of bioaccumulation data between different species and/or different tissues types have not yet been developed and comparison of tissue burdens among the species is not acceptable. Thus, it is essential that monitoring design elements be standardized to allow for comparisons among studies.

4.2.3 Analytical Methods Considerations

Questions to be considered during the choice of an appropriate analytical method include the parameters of interest, desired detection limits, sample size requirements or restrictions, methods of preservation, technical and practical holding times, and matrix interferences (especially from saline water). D'Elia et al. (1989) discuss the common analytical problems encountered during monitoring analyses of water samples.

Selection of appropriate methods should be based on a compromise between full-scan analyses, which are economical but cannot provide optimal sensitivity for all compounds, and alternative methods that are more sensitive for specific compounds but

can result in higher analytical costs. A list of analytical techniques is presented in Table 4-8. Table 4-9 shows which technique is appropriate for detecting a specific organic contaminant.

Dissolved Oxygen

The titrimetric, or Winkler, method is the first method of choice for the measurement of dissolved oxygen (USEPA, 1983a). The membrane electrode method (360.1) is recommended for samples containing interferents such as sulfur compounds, chlorine, free iodine, color, turbidity, or biological flocs, and also when continuous monitoring is planned (USEPA, 1983a).

Nutrients

Nutrients such as ammonia nitrogen, total Kjeldahl nitrogen, nitrate-nitrite nitrogen, and phosphorus are determined by spectrophotometric measurements using a segmented continuous flow analyzer, where samples and reagents are continuously added in sequence separated by air bubbles and pumped through glass tubing (USEPA, 1983a). Successive analyses can be accomplished in less time than would be required by manual methods because each analysis is not carried to completion, but is brought to the same stage of development and exposure by the timing of the stream flow through the system. Possible interferences include suspended solids, metal ions, and residual chlorine.

Ammonia nitrogen is determined by treating the samples with alkaline phenol and hypochlorite to produce indophenol blue, which is intensified with sodium nitroprusside. Standard solutions should be made up using substitute sea water to approximate the matrix of the samples (USEPA, 1983a). Total Kjeldahl nitrogen is defined as the sum of free ammonia and organic nitrogen compounds, which are converted to ammonium sulfate under the conditions of digestion (USEPA, 1983a). Sulfates react with nitrogen compounds of biological origin, but may not convert nitrogenous compounds of some industrial wastes. For the determination of nitrate-nitrite nitrogen, a filtered sample is passed through a granulated copper-cadmium column to reduce any nitrate to nitrite (USEPA, 1983a). The nitrite is then transformed to a highly colored azo dye, which is measured spectrophotometrically.

Total phosphorus is determined by heating the samples in the presence of sulfates, then cooling and measuring spectrophotometrically (USEPA, 1983a).

The fluorometric method for chlorophyll *a* is more sensitive than the spectrophotometric method. The sample is subjected to an excitation wavelength, and the fluorescence is measured at a second emission wavelength (APHA, 1989). The greatest uncertainty in the method is the choice of reference standard. High performance liquid

Table 4-8. List of Analytical Techniques

DISSOLVED OXYGEN	
• Winkler Titration	USEPA method 360.2
• Membrane Electrode	USEPA method 360.1
NUTRIENTS	
• Continuous Flow Spectrophotometry	
- Ammonia Nitrogen	USEPA method 350.1
- Total Kjeldahl Nitrogen	USEPA method 351.2
- Nitrate-Nitrite Nitrogen	USEPA method 353.2
- Total Phosphorus	USEPA method 365.4
• Fluorescence Spectrometry	
- Chlorophyll *a*	Standard Method 10200
• High Performance Liquid Chromatography (HPLC)	
TRACE METALS	
• Atomic Absorption Spectrophotometry (AAS)	USEPA 200 series methods
- flame	
- graphite furnace (GFAAS)	
- cold vapor	
- gaseous hydride (HYDAAS)	
• Inductively Coupled Plasma Emission Spectrometry (ICP)	USEPA method 200.7
ORGANICS	
• Gas Chromatography	USEPA 500/600 series methods
- with electron capture detection (GC/ECD)	
- with mass spectrometry (GC/MS)	
• Liquid Chromatography	
- with mass spectrometry (LC/MS)	

chromatography (HPLC) is the most accurate of the methods used for the analysis of chlorophyll (since all forms can be separately quantitated); however, it is also the most expensive.

Table 4-9. USEPA Organic Contamination Detection Techniques

USEPA Method[a]	Contaminant Type	Method
601	Purgeable Halocarbons	GC-ELCD
602	Purgeable Aromatics	GC-PID
603	Acrolein/Acrylonitrile	GC-FID and PID
604	Phenols	GC-FID
606	Phthalate Esters	GC-ECD
608	PCBs and Organochlorine Pesticides	GC-ECD
609	Nitroaromatics and Cyclic Ketones	GC-FID-ECD
610	Polycyclic Aromatic Hydrocarbons	HPLC-UV-FL or GC-FID
611	Haloethers	GC-ECD
614	Organophosphorous Pesticides	GC-FPD or NPD
624	Volatile Organics	GC-MS
625	Semivolatile Organics	GC-MS

[a] USEPA, 1983a.

Trace Metals

The choice of analytical method for trace metal analysis is determined by the required detection limit. Inductively coupled plasma emission spectrometry (ICP) allows the simultaneous measurement of several elements; however, the achievable detection limits are usually not as low as those obtained by graphite furnace or hydride atomic absorption spectrophometry (AA). The combination of AA and ICP is the recommended analytical method for detection of metals since no technique is best for all elements. Cold vapor AA is the recommended technique for the analysis of mercury.

Graphite furnace AA is more sensitive than flame AA or ICP but is more subject to matrix and spectral interferences. Such interferences result in potential quality control problems during the analyses and uncertainties in the resulting data. Because of the lower concentrations that can be seen by graphite furnace AA, particular caution must be taken with regard to laboratory contamination. The concentration of each element is determined by a separate analysis, making the analysis of a large number of contaminant metals both labor-intensive and relatively expensive compared to ICP. AA

methods may, however, be cost-effective for the analysis of a few metals over a large number of samples. It should be noted that "ultraclean" sampling and analytical techniques should be used for trace metal samples in open ocean waters.

Semivolatile Organic Compounds

Analysis of semivolatile organic compounds involves a solvent extraction of the sample, cleanup of the extract, gas chromatographic (GC) separation, and quantitation. There are two gas chromatography/mass spectrometry (GC/MS) options for detecting extractable organic compounds: internal standard and isotope dilution. The isotope dilution technique, which requires spiking the sample with a mixture of stable isotope-labeled analogs of the analytes, is recommended because reliable recovery corrections can be made for each analyte with a labeled analog or a chemically similar analog. This method is more expensive and less widely employed than the internal standard method, which is the current method of choice in EPA's Contract Laboratory Program. Mass spectrometry provides positive compound identification by comparison of both retention time and spectral patterns with standard compounds.

Many organic compounds can be identified by gas chromatography/electron capture detection (GC/ECD) analysis. GC/ECD provides greater sensitivity (lower detection limits) relative to GC/MS; however, GC/ECD does not provide positive compound identification. Since the identity of a compound is determined solely by matching retention time with that of a standard, confirmation of the initial identity of the compound on a second GC column is required for confidence in the reliability of the qualitative identification of the compound. When a compound is present at a high enough concentration, its identity should be confirmed by the use of GC/MS. GD/ECD is the method of choice for pesticides and PCBs. Glass capillary GC achieves a much higher degree of resolution in the analysis of the PCB congeners than the standard packed column methods; however, few labs regularly employ this specialized technique. The liquid chromatography/mass spectrometry (LC/MS) method is being developed for the detection and quantitation of nonvolatile organic compounds.

Volatile Organic Compounds

The purge-and-trap GC/MS technique is commonly employed for detecting volatile organic compounds in water. As in the case of semivolatiles, GC/ECD may be used to achieve lower detection limits, although this introduces a level of uncertainty to the qualitative identification of compounds. The isotope dilution technique is recommended if the Data Quality Objectives (DQOs) of the monitoring program require accurate quantitation of each compound. This technique, however, carries additional analytical time and expense.

4.2.4 QA/QC Considerations

Measurement data are no better than the planning that goes into setting data quality objectives, choosing appropriate sampling and analysis methodologies, and setting quality control criteria. An expert chemist and the references cited in this document should be used to develop a comprehensive QA plan.

Sample Collection

The primary criterion for an adequate sampling device is that it consistently collect undisturbed and uncontaminated samples. Water column sampling devices should be inspected for wear and tear leading to sample leakage upon ascent. It is prudent to have a backup sampler on board the survey vessel in case the primary sampler is found to be unsuitable during the cruise.

In the field, sources of contamination include sampling gear, lubricants and oils, engine exhaust, airborne dust, and ice used for cooling samples. If samples are designated for chemical analysis, all sampling equipment (e.g., siphon hoses, scoops, containers) must be made of noncontaminating material and must be cleaned appropriately prior to use. Potential airborne contamination such as stack gases and cigarette smoke should be avoided. Furthermore, samples and sampling containers should not be touched with ungloved fingers. Sampling containers and preservatives are summarized in Table 4-10.

Splitting water samples for ultraclean chemical analysis (for open ocean samples—especially for metals) and the analyses themselves should be conducted with noncontaminating tools under "clean room" conditions. Recommended container materials, sample sizes, preservation techniques, and analytical holding times should be determined before the sampling effort is undertaken.

Various types of blanks can be prepared and analyzed to identify possible sources of sample contamination. Field blanks are aqueous samples manipulated in the field. Rinsate blanks are aqueous samples that are poured over the sampling equipment and collected. Filtration blanks are aqueous samples drawn through the filtration apparatus. Trip blanks are packed in the sample coolers to detect cross-contamination of volatile organic compounds.

Blind duplicates, treated and identified as separate samples, may be sent to the same laboratory for analysis, or one of the pair may be sent to a "reference" laboratory for comparison. Standard reference material may be prepared and sent as a performance check to the laboratory. Depending on how these types of samples are handled, they may be used as a check for accuracy and/or precision.

Table 4-10. Sample Preservation and Storage Parameters

Analyte	Sample		Storage	
	Container[a]	Size	Preservative	Lifetime
Nutrients				
Ammonia	P,G	500 mL	H_2SO_4, pH<2[b]	28 days
Nitrate	P,G	100 mL	H_2SO_4, pH<2[b] refrigerate	48 hours
Nitrate + Nitrite	P,G	200 mL	H_2SO_4, pH<2[b]	28 days
Nitrite	P,G	100 mL	H_2SO_4, pH<2[b]	48 hours
Total or dissolved metals (except Hg)	P,G,TFE[c]	500 mL	HNO_3, pH<2	6 months
Total or dissolved Hg	G,TFE	250 mL	HNO_3, pH<2	28 days
Particulate metals	P,G	1 gal	-[d]	6 months
Semivolatiles	G	1 L	Cool, 4°C extraction	7 days to
Volatiles	G	40 mL	Cool, 4°C	7 days

[a] P = linear polyethylene, G = borosilicate glass, TFE = tetrafluoroethylene.

[b] It is recommended that samples be analyzed as soon as possible.

[c] If aliquot for Hg taken from this sample, cannot use linear polyethylene.

[d] Samples should be filtered as soon as possible and always within 24 hr.

Laboratory Analyses

Laboratory performance standards as measured by method QC protocols should be used to evaluate and select appropriate analytical methods. Changes in selected laboratory protocols should only be considered and/or approved if proposed procedures meet or exceed established performance criteria.

QA reports should describe the results of quantitative QC analyses, as well as other elements critical to accurate interpretation of analytical results. It is recommended that these reports be recorded and stored in a data base for future reference.

Trip blanks indicate whether any contamination occurred in the field or during shipping of samples. Rinsate blanks are used to check for contamination due to inadequate cleaning of field equipment.

Field splits, treated and identified as separate samples, may be sent to the same laboratory for analysis or one sample may be sent to a "reference" laboratory for comparison. Standard reference material may be placed in a sample container at the time of collection and sent "blind" to the laboratory.

Calibration standards should be analyzed at the beginning of sample analysis and should be verified at the end of each 12-hr shift during which analyses are performed. The concentrations of calibration standards should bracket the expected sample concentrations, or sample dilutions or sample handling modifications (i.e., reduced sample size) will be required.

Analysis of method or reagent blanks should be conducted to demonstrate the absence of contamination from sample handling in the laboratory. At least one method blank must be included with each batch of samples and should constitute at least 5 percent of all samples analyzed. All blanks should be free of detectable concentrations of the target compounds, or steps should be taken to remove the source of contamination before sample analysis proceeds.

Spike recovery analyses are required to assess method performance for the particular sample matrix. Spike recoveries serve as an indication of analytical accuracy, whereas analysis of standard reference materials (SRMs) can measure extraction efficiency. Commonly recommended control limits include 75-125 percent recovery for spikes and 80-120 percent recovery for the analysis of SRMs.

Replicates must be analyzed to monitor the precision of laboratory analyses. A minimum of 5 percent of the analyses should be laboratory replicates. Field duplicates should also be collected where practical. Common control limits are $\pm$20 relative percent difference between duplicates.

Currently, no universally accepted convention for reporting detection limits of analytical procedures exists. Table 4-11 lists definitions of various detection limits used by the American Chemical Society's Committee on Environmental Improvement (CEI). The IDL does not address possible blank contaminants or matrix interferences and is not a good standard for complex environmental matrices. The LOD and LOQ account for

blanks, but not matrix interferences. The MDL provides high statistical confidence but, like the LOQ, may be too stringent. Detection limits must be specified and reported to ensure adequate data quality and comparability between protocols.

4.2.5 Statistical Design Considerations

The statistical analysis of water chemistry data typically involves calculating summary statistics and testing for spatial and temporal trends. Summary conditions are useful for spatial displays (e.g., plume contours), load estimations, and general reporting. Testing for trends is useful for evaluating whether water chemistry conditions have improved or degraded over time or space. In comparison to other variables (e.g., see Bioaccumulation Methods), it is not common to collect replicated or composite data except for QC procedures. This is the usual case since most monitoring designs typically opt for collecting water chemistry data more frequently or at more stations.

Summary Statistics

To summarize data, the analyst typically estimates statistics for central tendency (e.g., mean, median) and variability (e.g., standard deviation, interquartile range) of grouped data. Uncertainty should be indicated by reporting estimates with confidence limits or

Table 4-11. Definitions for Selected Limits of Detection

Instrument Detection Limit (IDL)	The smallest signal above background noise that an instrument can detect reliably.
Limit of Detection (LOD)	The lowest concentration level that can be determined to be statistically different from the blank. The recommended value for LOD is 3σ, where σ is the standard deviation of the blank in replicate analyses.
Limit of Quantitation (LOQ)	The level above which quantitative results may be obtained with a specified degree of confidence. The recommended value for LOQ is 10σ, where σ is the standard deviation of the blank in replicate analyses.
Method Detection Limit (MDL)	The minimum concentration of a substance that can be identified, measured, and reported with 99 percent confidence that the analyte concentration is greater than zero. The MDL is determined from seven replicate analyses of a sample of a given matrix containing the analyte.

percentiles (Ward and Loftis, 1986). Alternatively, data may be summarized graphically with plots (e.g., box and whisker plots, time series plots, scatter plots, contours, profiles, etc.).

When developing summary statistics, it is important to use comparable data. It is recommended that data collected or analyzed with different methods not be combined unless a study comparing the different methods has been performed. An appropriate study would establish the level of comparability between methods.

For the purposes of estimating summary statistics, it is generally recommended that statistics not be used on left-censored data (i.e., data less than or equal to the detection limit). Porter et al. (1988) and Gilliom and Helsel (1986) provide a discussion on the implications of analyzing left-censored data and alternative procedures for estimating summary statistics when the data are censored.

Trend Detection

Prior to the collection of data, the statistical test (and significance level) used to analyze the data should be specified. By specifying the test to be used a priori, the monitoring plan can be designed to ensure that the correct type of data (e.g., spatial versus temporal coverage) is collected.

Monitoring programs can be evaluated using power analysis. The relative power of a sampling is more meaningful when the relative costs of implementing alternative designs are taken into consideration. Power-cost analyses are fundamental in selecting appropriate sample/replicate number, sample location, and sampling frequency (Ferraro et al., 1989).

For allocating samples and resources, Hirsch (1988) provides an approach for evaluating alternative sampling strategies for two-sample designs. This approach would be appropriate when comparing concentrations of a water chemistry variable from two different locations (e.g., an impaired and control area) or two different time periods (e.g., two different cruises) at the same location. As a general recommendation, equal resources (e.g., equal number of samples) should be allocated for each sampling period for two-sample designs (Hirsch, 1988).

4.2.6 Use of Data

Results of the chemical analyses can be used to monitor levels of pollutants both in the zone of initial dilution and in areas only minimally affected by the discharge. Sampling in this manner establishes spatial trends in the accumulation and transport of discharged pollutants.

The data can also be used as boundary and initial conditions for modeling work that may be used to map the transport and fate of various chemicals throughout the ecosystem under varying discharge scenarios. The modeling can lead to developing water quality standards and discharge guidelines. During the monitoring program, water chemistry data can be used to identify discharges that are not in compliance and to ensure that water quality standards are being maintained.

Monitoring of pollutant levels in the water column is a widely-accepted means of measuring the condition of the aquatic habitat. However, the singular use of pollutant loading data to assess the condition of the water column or to guide the decision-making process is not recommended. Data acquired from monitoring water contaminant levels, in conjunction with the water's physical properties, may be used to assess the health risks to human populations and the ecological risks to individuals, populations, and communities living in the water column. Monitoring of water column chemistry remains a powerful tool in the evaluation of spatial and temporal effects of anthropogenic and natural disturbance.

4.2.7 Summary and Recommendations

Rationale

- Monitoring water quality parameters will provide information on ambient water conditions and the potential for transport and persistence of contaminants from permitted discharges.

Monitoring Design Considerations

- The analytical methods must show appropriate selectivity, specificity, and sensitivity for the contaminants of concern.
- Sampling and sample-handling procedures should be well-defined and consistent so as not to compromise the integrity and representativeness of the samples.
- Sampling numbers and locations should be appropriate to the level of information required.

Analytical Methods Considerations

- The Winkler (titrimetric) method is the first method of choice for the measurement of dissolved oxygen unless the samples contain interferents such as sulfur compounds, chlorine, free iodine, color, turbidity, or biological flocs, or when continuous monitoring is planned. In those instances, a membrane electrode should be used.

- Nutrients such as ammonia nitrogen, total Kjeldahl nitrogen, nitrate-nitrite nitrogen, and phosphorus are determined by spectrophotometric measurements using a segmented continuous flow analyzer.
- The usual method for chlorophyll determination is fluorometric; however, the choice of method is determined by the need to separate the different forms. HPLC is the most accurate method, but it is also the most expensive.
- A combination of AA spectroscopy and ICP spectrometry is the recommended method for the detection of metals.
- Cold vapor AA spectroscopy is the recommended protocol for the detection of mercury.
- For monitoring programs where the most accurate quantitation is important, the isotope dilution GC/MS method is recommended for volatile and semivolatile organic compounds. GC/ECD is the method of choice for many organic chemicals (e.g., pesticides and PCBs).

QA/QC Considerations

- Calibration standards and blank, matrix spike, and replicate analyses are recommended quality control checks.
- A report describing the objectives of the analytical effort; methods of sample collection, handling, and preservation; details of the analytical method; problems encountered during the analytical process; any necessary modifications to the written procedures; and results of the QC analyses should be included with the quantitative data.

Statistical Design Considerations

- The analyst typically estimates statistics for central tendency (e.g., mean, median) and variability (e.g., standard deviation, interquartile range) of grouped data. Uncertainty should be indicated by reporting estimates with confidence limits or percentiles.
- When developing summary statistics, it is important to use comparable data. It is recommended that data collected or analyzed with different methods not be combined unless a study comparing the different methods has been performed.
- For the purpose of estimating summary statistics, it is generally recommended that statistics not be performed on left-censored data.
- Prior to the collection of data, the statistical test (and significance level) used to analyze the data should be specified.

- Monitoring programs can be evaluated using power analysis for detecting long-term (monotonic) trends in nutrient data. In general, the approach described there can be applied for other variables and statistical tests (e.g., monotonic or step-trend test).

Use of Data

- Monitor ambient levels of pollutants in the environment.
- Establish spatial and temporal trends in the accumulation and transport of pollutants discharged into ambient waters.
- Calibrate and verify mathematical models.
- Develop water quality standards for receiving waters.
- Identify noncompliant discharges.

4.3 SEDIMENT CHEMISTRY

Monitoring of pollutant levels in sediments is a widely-accepted means of measuring the condition of the benthic habitat and is a powerful tool in the evaluation of spatial and temporal effects of anthropogenic and natural disturbance. Sediment pollutant loading data should be used, however, in conjunction with other tools to assess the condition of the benthic habitat or to guide the decision-making process.

4.3.1 Rationale

Of the 10 guidelines used to determine unreasonable degradation or irreparable harm, the following can be assessed by evaluating sediment chemistry:

- Quantities, composition, and potential bioaccumulation or persistence of the pollutants to be discharged;
- Potential transport of the pollutants by biological, physical, or chemical processes; and
- Potential direct or indirect impacts on human health.

The sediments represent a potential sink for many chemical contaminants in the marine environment (USEPA, 1989e). The objective of monitoring bulk sediment chemistry is to detect sediment toxicity and to describe spatial and temporal changes in sediment pollutants. The results may be used to monitor rates of change following discharge initialization, to evaluate the condition of benthic habitats, and to provide early warnings of potential impacts to the marine ecosystem.

4.3.2 Monitoring Design Considerations

Selection of Analytes

The chemicals that should be included in the monitoring program are those chemicals known or suspected to be in the discharge, as well as possible by-products. Although potentially toxic compounds in the discharge may not be fully known, the objectives of the program must be clearly defined. It may be necessary at the beginning of the program to conduct an analysis for all chemicals on the EPA-defined Priority Pollutant, Hazardous Substance, or Target Compound/Analyte Lists to determine which are present; however, detection in the environment does not always correlate with biological risks and effects. Limitations in analytical methodology, modeling techniques, and toxicological data restrict the usefulness of the resulting data. In some cases, levels of contaminants that are of concern to the health of organisms are below the lowest limit of detection of the best available analytical methodology.

Sampling Considerations

Selection of sampling locations and choice of sampling techniques are crucial decisions to be made before the sampling effort is initiated. *Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound* (USEPA, 1986-1991) contains discussions of quality assurance requirements and sampling considerations that are applicable to sediment quality monitoring. Sampling must incorporate sites within the zone of initial dilution, extending out to areas of low contaminant concentration and further to unaffected areas to obtain the complete spatial variability. A summary of sample containers and handling guidelines is presented in Table 4-12.

Sediment Sampling Devices

The protocols required to collect an acceptable surficial sediment sample for subsequent measurement of chemical variables have generally been neglected. In fact, sampling crews have been given wide latitude in how samples are collected. Because sample collection protocols influence all subsequent laboratory and data analysis, however, it is essential that sediment samples be collected using acceptable and standardized techniques. Sediment sampling devices are the same regardless of whether the samples are collected for chemistry, grain size, or benthic infauna analyses. Consequently, enough sediment may be collected in the same grab or core for all three analyses. Equipment selection depends in large part on the use of the sample collected. Consideration should also be given to water depth, currents, ocean bottom characteristics, and degree of homogeneity and representativeness of sampling.

Collection of undisturbed sediment requires that the sampler:

- Create a minimal bow wake when descending;
- Form a leakproof seal when the sediment sample is taken;
- Prevent winnowing and excessive sample disturbance when ascending; and
- Allow easy access to the sample surface in order that undisturbed subsamples may be taken (ASTM, 1991).

Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes. It is best to use a sampler with a weight adjustment to enable the modification of penetration depths.

Several types of devices can be used to collect sediment samples: dredges, grabs, and box corers (McIntyre et al., 1984). There are many variations to these types of sampling device. Many of these devices sample the benthic habitat in a unique manner (Table 4-13). Accordingly, conducting comparisons between data collected using different devices is inadvisable.

Table 4-12. Sampling Containers, Preservation Requirements, and Holding Times for Sediment Samples

Contaminant	Container[a]	Preservation	Holding Time
Acidity	P,G	Cool, 4°C	14 days
Alkalinity	P,G	Cool, 4°C	14 days
Ammonia	P,G	Cool, 4°C	28 days
Sulfate	P,G	Cool, 4°C	28 days
Sulfide	P,G	Cool, 4°C	28 days
Sulfite	P,G	Cool, 4°C	48 hours
Nitrate	P,G	Cool, 4°C	48 hours
Nitrate-nitrite	P.G	Cool, 4°C	28 days
Nitrite	P,G	Cool, 4°C	48 hours
Oil and grease	G	Cool, 4°C	28 days
Organic carbon	P,G	Cool, 4°C	28 days
Metals			
Chromium VI	P,G	Cool, 4°C	48 hours
Mercury	P,G		28 days
Metals, except above	P,G		180 days
Organic Compounds			
Extractables (including phthalates, nitrosamines, organochlorine pesticides, PCBs, nitroaromatics, isophorone, polynuclear aromatic hydrocarbons, haloethers, chlorinated hydrocarbons, and TCDD)	G, Teflon-lined cap	Cool, 4°C	7 days (until extraction) 40 days (after extraction)
Extractables (phenols)	G, teflon-lined cap	Cool, 4°C	7 days (until extraction) 40 days (after extraction)
Purgeables (halocarbons and aromatics)	G, Teflon-lined septum	Cool, 4°C	7 days
Purgeables (arolein and acrylonitrite)	G, Teflon-lined septum	Cool, 4°C	3 days
Orthophosphate	P,G	Cool, 4°C	48 hours
Pesticides	G, Teflon-lined cap	Cool, 4°C	7 days (until extraction) 40 days (after extraction)
Phenols	P,G	Cool, 4°C	28 days
Phosphorus (elemental)	G	Cool, 4°C	48 hours
Phosphorus, total	P,G	Cool, 4°C	28 days
Chlorinated organic compounds	G, Teflon-lined cap	Cool, 4°C	7 days (until extraction) 30 days (after extraction)

[a]Polyethylene (P) or glass (G)
SOURCE: ASTM, 1991.

Table 4-13. Summary of Bottom Sampling Equipment[a]

Device	Use	Advantages	Disadvantages
Fluorocarbon plastic or glass tube	Shallow wadable waters or deep waters if SCUBA available. Soft or semiconsolidated deposits.	Preserves layering and permits historical sudy of sediment deposition. Rapid-samples immed-iately ready for laboratory shipment. Minimal risk of contamination.	Small sample size requires repetitive sampling.
Hand corer with removable fluorocarbon plastic or glass liners	Same as above except more consolidated sedi-ments can be obtained.	Handles provide for greater ease of substrate penetration. Above advantages.	Careful handling necessary to prevent spillage, Requires removal of liners before repetitive sampling. Slight risk of metal contamination from barrel and core cutter.
Box corer	Same as above.	Collection of large undisturbed sample allowing for subsampling.	Hard to handle.
Gravity corers, that is, Phleger corer	Semiconsolidated sediments.	Low risk of undisturbed sample contamination. Maintains sediment integrity relatively well.	Careful handling necessary to avoid sediment spillage. Small sample, requires repetitive operation and removal of liners. Time-consuming.
Young grab (fluoro-carbon plastic- or Kynar-lined modi-fied 0.1 m^2 Van Veen)	Lakes and marine areas.	Eliminates metal contamination. Reduces pressure wave.	Expensive. Requires winch.
Ekman or box dredge	Soft to semisoft sediments. Can be used from boat, bridge, or pier in waters of various depths.	Obtains a larger sample than coring tubes. Can be subsampled through box lid.	Possible incomplete jaw closure and sample loss. Possible shock wave, which may disturb the fine sediments ("fines"). Metal construction may introduce contaminants. Possible loss of "fines" on retrieval.
Ponar grab sampler	Useful on sand, silt, or clay.	Most universal grab sampler. Adequate on most substrates. Large sample obtained intact, permitting subsampling.	Shock wave from descent may disturb "fines." Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.

Table 4-13. (continued)

Device	Use	Advantages	Disadvantages
BMH-53 piston corer	Waters of 4-6 ft depth when used with extension rod. Soft to semiconsolidated deposits.	Piston provides for greater sample retention.	Cores must be extruded on site to other containers. Metal barrels introduce risk of metal contamination.
Van Veen	Useful on sand, silt, or clay.	Adequate on most substrates. Large sample obtained intact, permitting subsampling.	Shock wave from descent may disturb "fines." Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.
BMH-60	Sampling moving waters from a fixed platform.	Streamlined configuration allows sampling where other devices could not achieve proper orientation.	Possible contamination from metal construction. Subsampling difficult. Not effective for sampling fine sediments.
Petersen grab sampler	Useful on most substrates.	Large sample; can penetrate most substrates.	Heavy, may require winch. No cover lid to permit subsampling. All other disadvantages of Ekman and Ponar.
Shipek grab sampler	Used primarily in marine waters and large inland lakes and reservoirs.	Sample bucket may be opened to permit subsampling. Retains fine-grained sediments effectively.	Possible contamination from metal construction. Heavy, may require winch.
Orange-peel grab Smith-McIntyre grab	Useful on most substrates.	Designed for sampling hard substrates.	Loss of "fines." Heavy, may require winch. Possible metal contamination.
Scoops, drag buckets	Various environments depending on depth and substrate.	Inexpensive, easy to handle.	Loss of "fines" on retrieval through water column.

[a]Comments represent subjective evaluations.
SOURCE: ASTM, 1991.

Grab samplers

Grabs are capable of consistent sampling of bottom habitats. Depending on the size of the device, areas from 0.02 to 0.5 m^2 and depths ranging from 5 to 15 cm may be sampled. Limitations of grab samplers include :

- Variability among samples in penetration depth depending on sediment properties;
- Oblique angles of penetration, which result in varying penetration depths within a sample; and
- Folding of the sample, which results in the inability to section the sample and the loss of information concerning the vertical structure in the sediments.

The careful use of these devices, however, will provide quantitative data. Grab samplers are the tools of choice for a number of marine monitoring programs (Fredette et al., 1989; USEPA, 1986-1991).

Core samplers

Box corers use a surrounding frame to ensure vertical entry; therefore, vertical sectioning of the sample is possible (USEPA, 1986-1991). These devices are capable of maximum penetration depths of 15 cm and may collect volumes 5 to 10 times those of grab samplers. Limitations of box corers include :

- Large size and weight, which require the use of cranes or winches and a large vessel for deployment;
- Higher construction expenses; and
- Lack of calibration studies to permit comparisons to grab samples.

The Hessler-Sandia box corer uses dividers to section the core into subsections, facilitating subsampling of the core. Box corers are recognized as the tools of choice for maximum accuracy and precision when sampling soft-bottom habitats.

Collection of sediments and collection of benthic organisms should be done concurrently to mitigate the costs of field sampling and to permit sound correlation, regression, and multivariate analyses. Therefore, it is recommended that the sampling device also be suitable for benthic sampling. Grab and core sampling devices permit adequate sampling of both sediment and benthic infaunal communities with one sampling device.

Sample Depth

It is recommended that the upper 2 cm of the sediment column be examined to characterize surficial sediments. Although the 2 cm specification is arbitrary, it does ensure that relatively recent sediments are sampled, that adequate volumes of sediments are readily obtained for laboratory analyses, and that data from different studies can be compared.

Sampling of depths other than 2 cm or vertical stratification of deeper sediment cores may be appropriate, depending on the objectives of the monitoring program and the rate of sediment accumulation. For example, if information concerning only the most recent sediment contamination is required, examination of the upper 1 cm may be appropriate. Stratification of deeper cores will provide historical data of sediment contaminant levels and depositional events. Comparison of data from studies analyzing different sediment depths is not advised. If the potential for bioaccumulation of contaminants in infaunal organisms is a concern, sampling to the depth of the anoxic layer is desirable. Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes.

Total Organic Carbon and Acid Volatile Sulfides Normalization

Total organic carbon (TOC) and acid volatile sulfides (AVS) are considered by many to be the most important parameters in defining organic and metal concentrations in sediments. Toxic hydrophobic contaminant concentrations have been found to be related to the TOC content of the sediment (Karickhoff et al., 1979). Likewise, toxic metals concentrations have been found to be related to the AVS concentration of the sediment (DiToro et al., in press a, b). The AVS pool is available to bind metals, thereby reducing their bioavailability.

Normalizations of sediment contaminant concentrations to TOC and AVS have been conducted to estimate the bioavailability of inorganic and organic contaminants. Furthermore, these normalizations have been made to account for some of the variability found in bioaccumulation rates and biological assemblages. TOC/lipid normalized accumulation factors (AF) have also been used to predict tissue residue concentrations (Ferraro et al., 1990; Lake et al., 1987). AVS will be used in a similar manner to assist in the development of accumulation factors for metals.

Normalization based on AVS concentrations is considered a potentially useful tool for assessing the bioavailability of certain divalent metals (e.g., Cd and Ni). However, only a few laboratories can perform these analyses at present and the procedure has not undergone any round robin verification. In addition, sampling for AVS is very subject to error because contact with air can greatly affect results. Although AVS normalizations represent a promising tool for evaluation of sediment metals data, general use in estuarine monitoring programs may not yet be practical.

These normalizations assume the following:

- Organic contaminants partition predominantly to sediment organic carbon; metal contaminants partition predominantly to sediment AVS.
- Rapid steady-state kinetics of contaminants.

Development of standardized methods for measuring TOC and AVS in the laboratory are required before normalized contaminant concentrations may be compared among studies.

The decision to normalize for TOC and AVS will depend on monitoring program objectives. For example, if the objective is to identify the "footprint" of a discharge, normalization may not be appropriate; if the monitoring objective is to identify "hot spots"—i.e., those areas where bioavailable contaminants represent a risk to human and ecological health—normalization may be justified.

Selection of Sampling Period

Sediment transport processes can vary on a seasonal scale, while ocean discharges are generally constant over time. Therefore, sediment contaminant levels may display a seasonal pattern in accumulation rates. Sediment processes are associated with seasonal patterns of benthic turbulent mixing and sediment transport phenomena. The frequency of sampling should take into account the expected rate of change in sediment contaminant concentration.

Because of seasonal variations, it is recommended that direct comparisons between samples collected during different times of the year be avoided. Studies investigating interannual variation in the concentrations of sediment contaminants should be conducted during the same season (preferably the same month) each year. Furthermore, it is recommended that sediments be sampled when contaminant concentrations are expected to be at their highest levels in order to evaluate worst-case scenarios.

Seasonal comparisons within stations, however, can be valid and useful because (1) seasonal water column changes can affect sediment chemistry (e.g., anoxia can affect AVS and metal bioavailability); (2) seasonal fluctuations in the presence, abundance, and activity of infauna can lead to changes in bioturbation and thereby the chemistry of surficial sediments; and (3) seasonal changes in discharge activity can lead to changes in sediment loadings (e.g., in Alaskan waters, where ice might preclude discharge during winters, one might expect chemical concentrations in sediments to be low in spring and higher in fall).

4.3.3 Analytical Methods Considerations

Questions to be considered during the choice of an appropriate analytical method include the parameters of interest, desired detection limits, sample size requirements or restrictions, methods of preservation, technical and practical holding times, and matrix interferences. Several USEPA documents (e.g., 1986a and 1986-1991) discuss the common analytical problems encountered during monitoring analyses of sediment samples. It will frequently be necessary to use methods other than those currently approved by EPA to achieve a desired sensitivity in a marine environment. However, alternative methods should be considered only when they have been demonstrated to provide a level of precision and accuracy equivalent to or exceeding that of the EPA-approved method. Any proposed changes in analytical methods should be reviewed by analysts with experience in marine waters.

Chemical Residue Analyses

Several factors determine achievable detection limits for a specific contaminant, regardless of analytical procedure. These factors include:

- Sample size; 50-100 g (wet weight) with a minimum final dilution volume of 0.5 mL is considered adequate (USEPA, 1986-1991).
- Presence of interfering substances.
- Range of pollutants to be analyzed; an optimal method may be developed without regard to potential effects on other parameters.
- Level of confirmation — qualitative (e.g., presence or absence) or quantitative (e.g., residue concentrations) analyses.
- Level of pollutant found in the field and in analytical blanks.

A list of analytical procedures and USEPA method numbers is given in Table 4-14. Selection of appropriate methods should be based on a trade-off between full-scan analyses, which are economical but cannot provide optimal sensitivity for some compounds, and alternative methods that are more sensitive for specific compounds but can result in higher analytical costs. Table 4-15 lists which technique is appropriate for detecting a specific contaminant.

Metals and Metalloids

Trace element analyses by inductively coupled plasma emission spectrometry (ICP) allow for several elements to be measured simultaneously. Detection limits of ICP for most metals are generally comparable to those achieved by graphite furnace atomic absorption spectrophotometry (GFAA); however, detection limits for several metals (e.g., arsenic, selenium, and mercury) are significantly lower using atomic absorption spectrophotometry (AA).

Table 4-14. List of Analytical Techniques (USEPA, 1986a)

METALS/METALLOIDS

- Atomic Absorption Spectrophotometry (AA)
  - flame — USEPA method 7000 series
  - graphite furnace (GFAA)
  - cold vapor — USEPA method 7470
  - gaseous hydride (HYDAA) — USEPA methods 7060 and 7740
- Inductively Coupled Plasma Emission Spectrometry (ICP) — USEPA method 6010

ORGANICS

- Gas Chromatography (GC)
  - with electron capture detection (GC/ECD) — USEPA method 8080
  - with mass spectrometry (GC/MS) — USEPA methods 8240 and 8270

Pollutants Tested for by Following Techniques:

ICP	AA
Al, Sb, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, K, Se, Si, Ag, Na, Tl, V, and Zn	Al, Sb, As, Ba, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Hg, Mo, Ni, Se, Ag, Tl, Sn, V, and Zn

SOURCE: USEPA, 1986a.

The combination of AA and ICP is the recommended analytical method for detection of metals and metalloids since no technique is best for all elements (USEPA, 1986a). Cold vapor AA analysis is the recommended technique for mercury (USEPA, 1986a). Digestion methods for sediment samples are reviewed by Plumb (1981). The EPA Contract Laboratory Program requires the use of nitric acid and hydrogen peroxide (USEPA, 1990f).

GFAA is more sensitive (i.e., lower detection limits) than flame AA or ICP, but it is more subject to matrix and spectral interferences. GFAA requires particular caution with regard to laboratory contamination. The concentration of each element is determined by

Table 4-15. USEPA Organic Contaminant Detection Techniques

USEPA Method[a]	Contaminant Type	Method
8010	Purgeable Halocarbons	GC-ELCD
8015	Purgeable Nonhalogenated Volatiles	GC-FID
8020	Purgeable Aromatics	GC-PID
8021	Purgeable Organics	GC-FID and GC-PID
8030	Acrolein/Acrylonitrile	GC-FID and PID
8040	Phenols	GC-FID
8060	Phthalate Esters	GC-ECD
8080	PCBs and Organochlorine Pesticides	GC-ECD
8090	Nitroaromatics and Cyclic Ketones	GC-FID-ECD
8100	Polycyclic Aromatic Hydrocarbons	HPLC-UV-FL or GC-FID
8110	Haloethers	GC-ECD
8140	Organophosphorous Pesticides	GC-FPD or NPD
8150	Chlorinated Herbicides	GC-ECD or ELCD
8240	Volatile Organics	GC-MS
8270	Semivolatile Organics	GC-MS

[a] USEPA, 1986e.

a separate analysis, making the analysis of a large number of contaminant metals both labor-intensive and relatively expensive compared to ICP. AA methods may, however, be cost-effective for the analysis of a few metals over a large number of samples.

Semivolatile Organic Compounds

Analysis of semivolatile organic compounds involves a solvent extraction of the sample, cleanup of the characteristically complex extract, and gas chromatographic (GC) analysis and quantitation. There are two gas chromatography/mass spectrometry (GC/MS) options for detecting extractable organic compounds: internal standard and isotope dilution. The isotope dilution technique, which requires spiking the sample with a mixture of stable isotope-labeled analogs of the analytes, is recommended because reliable recovery corrections can be made for each analyte with a labeled analog or a chemically similar analog. The isotope dilution method is more expensive and less widely employed than the internal standard method, which is the current method of choice in the Contract Laboratory Program. Mass spectrometry provides positive compound identification by comparison of both retention time and spectral patterns with

standard compounds. This technique, however, lacks the sensitivity to detect levels of most trace contaminants in marine systems. Alternative methods providing greater sensitivity are available but are compound-specific and, therefore, more expensive.

The identification of many organic compounds can be made by gas chromatography/ electron capture detection (GC/ECD) analysis. GC/ECD provides greater sensitivity (lower detection limits) relative to GC/MS; however, GC/ECD does not provide positive compound identification. Since the identity of a compound is determined solely by matching retention time with that of a standard, confirmation of the initial identity of the compound on a second GC column is required for confidence in the reliability of the qualitative identification of the compound. When a compound is present at a high enough concentration, its identity should be confirmed by the use of GC/MS. GC/ECD is the method of choice for pesticides and PCBs. Glass capillary GC achieves a much higher degree of resolution in the analysis of PCB congeners than the standard packed column methods; however, few laboratories regularly employ this specialized technique.

The detection and concentration of PCBs can be more accurately determined using comparison mixtures other than the standard industrial Aroclor mixtures. Evaluations of PCB assemblages in environmental samples by quantification as Aroclors or total PCBs are of limited accuracy due to environmental degradation and differential affinities of PCB congeners for different environmental compartments. A more meaningful evaluation can be accomplished by quantification by PCB isomer groupings (dependent on the number of chlorine atoms per molecules). This has the advantage of indicating the relative concentrations of the groups containing the most toxic and bioaccumulating congeners (McFarland et al., 1986). An alternative method of analysis is to test for the individual PCB congeners. Using congener-specific methods instead of Aroclor standard mixtures provides more accurate identification and quantification and eliminates the necessity of subjective decisions on the part of the analyst (Eganhouse, 1990). However, beside being more expensive and time-consuming, the compatibility of these enhanced detection methods with the standard Aroclor method is an important consideration for monitoring programs with existing historical data. All other organic compound groups are recommended for analysis by GC/MS (USEPA, 1986a).

Volatile Organic Compounds

The purge-and-trap GC/MS technique is employed for detecting volatile organic compounds in water. A successful variation for detection of volatile organic residues in sediments involves a device that vaporizes volatile organic compounds from the sediment sample under vacuum and then condenses the volatiles in a super-cooled trap (Hiatt, 1981). The trap is then transferred to a purge-and-trap device, where it is treated as a water sample. The heated purge-and-trap technique, employed in the Contract Laboratory Program, involves the addition of a weighed amount of sediment to a known volume of water. As the chamber is heated, inert gas is bubbled through the suspension to sweep volatile organics out of the sediment. The isotope dilution option is

recommended if the data quality objectives (DQOs) of the monitoring program require accurate quantitation of each compound. This option involves additional analysis time and expense.

Sediment Toxicity Tests

Toxicity tests may be necessary if the sediment contains contaminants that could result in an adverse impact on the benthic environment and/or the water column. Tests with whole sediment are used to determine effects on benthic organisms. Tests with suspensions of the sediment are used to determine effects on water-column organisms. Test organisms should be representative of sensitive organisms existing in the vicinity of the actual site. Mortality of a certain percent of the organisms in the laboratory test does not imply that the population of the species around the site would decline by the same percent; however, results can be compared with reference-sediment results to determine whether the site sediment has higher toxicity (EA and Battelle, 1990).

An introductory guide to toxicity testing is presented in Part 8000 of APHA (1989). Most of the procedures currently used for sediment toxicity testing are based on USEPA/COE (1977). Additional discussion and procedures can be found in the draft testing manual for dredged material (EA and Battelle, 1990) and in USEPA (1986-1991) and Swartz et al. (1982, 1985).

Acute toxicity tests are conducted to determine the immediate effects of short-term exposures under specific conditions, providing little information about possible delayed effects. It can be difficult to apply laboratory results to field situations because of the fact that motile organisms might avoid exposure when possible and toxicity to benthic organisms is dependent on field-specific sediment characteristics, the dynamics of equilibrium partitioning, and routes of exposure. ASTM (1990) proposes standard guidelines for conducting 10-day static toxicity tests.

4.3.4 QA/QC Considerations

Measurement data are no better than the planning that goes into setting data quality objectives, choosing appropriate sampling and analysis methodologies, and setting quality control criteria. An expert chemist and the references cited in this document should be used to develop a comprehensive QA plan.

Sample Collection

The primary criterion for an adequate sampler is that it consistently collect undisturbed and uncontaminated samples. The sampling device should be inspected for wear and tear leading to sample leakage during ascent. It is recommended that a backup sampler be available on board the survey vessel in case the primary sampler is lost or becomes inoperable during the cruise.

In the field, sources of contamination include sampling gear, lubricants and oils, engine exhaust, airborne dust, and ice used for cooling samples. If samples are designated for chemical analysis, all sampling equipment (e.g., siphon hoses, scoops, containers) must be made of noncontaminating material and must be cleaned appropriately prior to use. Potential airborne contamination such as stack gases and cigarette smoke should be avoided. Furthermore, samples and sampling containers should not be touched with ungloved fingers.

The following sample acceptability criteria should be satisfied (USEPA, 1986-1991):

- The sampler is not overfilled with sample so that the sediment surface is pressed against the top of the sampler.
- Overlying water is present, indicating minimal leakage.
- The overlying water is not excessively turbid, indicating minimal sample disturbance.
- The sediment surface is relatively flat, indicating minimal disturbance or winnowing (Figure 4-1).

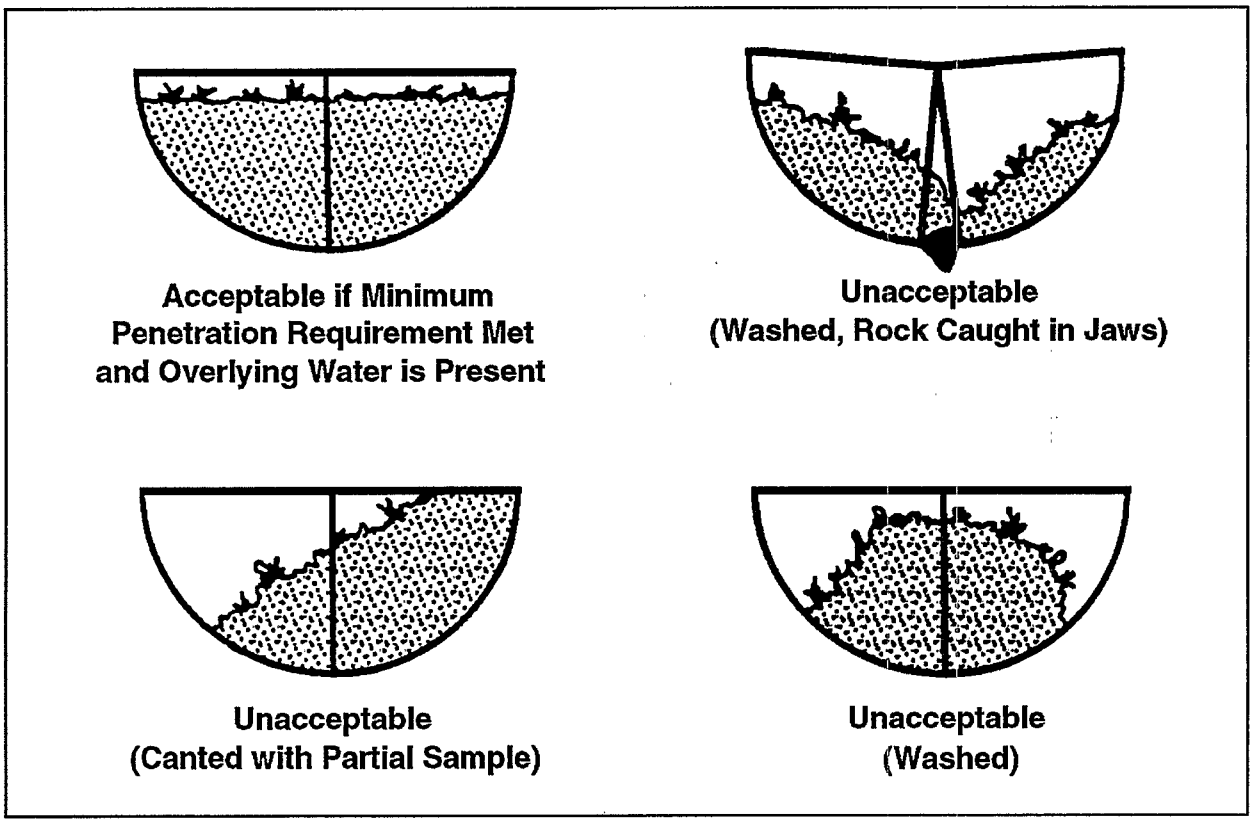


Figure 4-1. Examples of Acceptable and Unacceptable Samples

- The desired penetration depth is achieved.

If the sample does not meet all the criteria, it should be rejected.

Splitting of sediment samples for chemical analyses should only be conducted with noncontaminating tools under "clean room" conditions. Recommended container materials, sample sizes, preservation techniques, and analytical holding times should be determined before the sampling effort is undertaken.

For analyses of metals, samples should be frozen and kept at -20°C. Although specific holding times have not been recommended by EPA, a maximum of 6 months (28 days for mercury) would be consistent with those for aqueous samples (Table 4-12).

For analyses of volatile compounds, samples should be stored in the dark at 4°C (USEPA, 1986-1991; USEPA, 1987c). Analyses of volatile compounds should be performed within 7 days of collection as recommended by EPA. If extractions of semivolatile compounds will not be performed within 7 days, freezing of the samples at -20°C is advised. EPA has not established holding times for frozen samples. A general guideline of a maximum of 6 months would be consistent with that for water samples (USEPA, 1986-1991).

Samples for determination of TOC or AVS should be analyzed as soon as possible. If not analyzed immediately, TOC samples should be refrigerated and their pH brought below 2 by addition of H_2SO_4. Acid volatile sulfides should be stored in airtight containers under an inert atmosphere and analyzed as soon as possible.

Various types of blanks can be prepared and analyzed to identify possible sources of sample contamination. Field blanks are aqueous samples manipulated in the field. Rinsate blanks are aqueous samples that are poured over the sampling equipment and collected. Trip blanks are packed in the sample coolers to detect cross-contamination of volatile organic compounds.

Blind duplicates, treated and identified as separate samples, may be sent to the same laboratory for analysis, or one of the pair may be sent to a "reference" laboratory for comparison. Standard reference material may be prepared and sent as a performance check to the laboratory. Depending on how these types of samples are handled, they may be used as a check for accuracy and/or precision.

Laboratory Analyses

Laboratory performance standards as measured by method QC protocols should be used to evaluate and select appropriate analytical methods. Changes in selected laboratory protocols should be considered and/or approved only if proposed procedures meet or exceed established performance criteria. Tables 4-16 and 4-17 provide brief summaries of QC analyses for laboratory work.

QA reports should describe the results of quantitative QC analyses, as well as other elements critical to accurate interpretation of analytical results. It is recommended that these reports be recorded and stored in a database for future reference.

Table 4-16. Summary of Quality Control Analyses

Analysis Type	Recommended Frequency of Analysis
Surrogate Spikes	Required in every organic sample - minimum 3 neutral, 2 acid spikes, plus 1 spike for pesticide/PCB analyses, and 3 spikes for volatiles. Isotope dilution technique (i.e., with all available labeled surrogates) is recommended for full-scan analyses and to enable recovery corrections to be applied to data.
Method Blank	One per extraction batch (semivolatile organics). One per extraction batch or one per 12-hour shift, whichever is more frequent (volatile organics).
Standard Reference Materials	<50 samples: one per set of samples submitted to lab. ≥50 samples: one per 50 samples analyzed.
Matrix Spikes	Not required if complete isotope dilution technique is used. <20 samples: one per set of samples submitted to lab. ≥20 samples: 5 percent of total number of samples.
Spiked Method Blanks	As many as required to establish confidence in method before analysis of samples (i.e., when using a method for the first time or after any method modification).
Analytical Replicates	<20 samples: one per set of samples submitted to lab. ≥20 samples: 5 percent of total number of samples.
Field Replicates	At the discretion of the project coordinator.

Table 4-17. Summary of Warning and Control Limits for Quality Control Sample

Analysis Type	Recommended Warning Limit	Recommended Control Limit
Surrogate Spikes	10 percent recovery	50 percent recovery
Method Blank		
Phthalate, Acetone	30 percent of the analyte	5 µg total or 5 percent of the analyte
Other Organic Compounds	1 µg total or 5 percent of the analyte	2.5 µg total or 5 percent of the analyte
Standard Reference Materials	95 percent confidence interval	95 percent confidence interval for certified reference material
Matrix spikes	50-65 percent recovery	50 percent recovery
Spiked Method Blanks	50-65 percent recovery	50 percent recovery
Analytical Replicates	--	±100 percent coefficient of variation
Field Replicates	--	--
Ongoing Calibration	--	25 percent of initial calibration

Calibration standards should be analyzed at the beginning of sample analysis and should be verified at the end of each 12-hour shift during which analyses are performed. The concentrations of calibration standards should bracket the expected sample concentrations, or sample dilutions or sample handling modifications (i.e., reduced sample size) will be required.

Analysis of methods or reagent blanks should be conducted to demonstrate the absence of contamination from sample handling in the laboratory. At least one method blank must be included with each batch of samples and should constitute at

least 5 percent of all samples analyzed. All blanks should be free of detectable concentrations of the target compounds, or steps should be taken to remove the source of contamination before sample analysis proceeds.

Spike recovery analyses are required to assess method performance for the particular sample matrix. Spike recoveries serve as an indication of analytical accuracy, whereas analysis of standard reference materials (SRMs) can measure extraction efficiency. Commonly recommended control limits include 75-125 percent recovery for spikes and 80-120 percent recovery for the analysis of SRMs.

Replicates must be analyzed to monitor the precision of laboratory analyses. A minimum of 5 percent of the analyses should be laboratory replicates. Field duplicates should also be collected where practical. Common control limits are ±20 relative percent difference between duplicates.

Currently, no universally accepted convention for reporting detection limits of analytical procedures exists. Table 4-11 in the preceding section lists definitions of various detection limits used by the American Chemical Society's Committee on Environmental Improvement (CEI). The IDL does not address possible blank contaminants or matrix interferences and is not a good standard for complex environmental matrices. The LOD and LOQ account for blanks, but not matrix interferences. The MDL provides high statistical confidence but, like the LOQ, may be too stringent. Detection limits must be specified and reported to ensure adequate data quality and comparability between protocols.

4.3.5 Statistical Design Considerations

Statistical strategies may mitigate the high costs of collecting sufficient samples of sediment. Power analyses can affect the strategy of compositing samples and can often lead to a cost-effective monitoring design strategy.

Composite Sampling

Composite sediment sampling consists of mixing samples from two or more replications collected at a particular location over time or collected at several locations at the same time. The analysis of a composite sample provides an estimate of an average contaminant concentration for the locations composing the composite sample. Advantages of the composite sampling strategy are:

- It provides a cost-effective strategy when individual chemical analyses are expensive.
- It results in a more efficient estimate of the mean at specified sampling locations.

Because of the reduced sample variance, composite sampling results in a considerable increase in statistical power. If the primary objective of a monitoring program is to determine differences in sediment contaminant concentrations among sampling locations, composite sampling is an applicable strategy.

Space-bulking consists of sampling from several locations and combining sediment samples into one or more composite samples. Time-bulking involves taking multiple samples over time from a single location and compositing these samples. Space- and time-bulking strategies should be used judiciously since significant information concerning spatial and temporal heterogeneity may be lost.

Composite sampling is not recommended if the objective of the monitoring program is based on water quality-based criteria with specified sediment contaminant concentration limits since this sampling method does not detect the true range of sediment contaminant concentrations in the environment. The adoption of composite strategies will depend on the objectives of individual monitoring programs.

Statistical Power

Statistical power increases with an increase in the number of sediment subsamples in each replicate composite sample. However, with the addition of successive subsamples to each composite, a diminished return of statistical power exists. For composites of greater than 10 replications, the increase of power is negligible given typical levels of data variability.

To improve the power of a statistical test, while keeping the significance level constant, the sample size should be increased. Because of constraints in cost and time imposed by the monitoring program, however, this option may not be available. Power analyses have shown that for a fixed level of sampling effort, a monitoring program's power is generally increased by collecting more replicates at fewer locations. The number and distribution of sampling locations required to evaluate the effectiveness of the monitoring program will be depend on the size and complexity of the discharge.

4.3.6 Use of Data

The results may be used to monitor and evaluate the condition of benthic habitats and to provide early warnings of potential impacts to the ecosystem. The data generated from monitoring of the pollutant levels in estuarine sediments should be used in conjunction with those collected from other physical and biological monitoring. Subsequent analyses of health risks to human populations and ecological risks to benthic individuals, populations, and communities may be performed.

Sediment pollutant loading data should be used in combination with other monitoring methods to assess the condition of the benthic habitat or to guide the decision-making process. Data acquired from monitoring sediment contaminant levels, in conjunction with the sediment's physical properties, may be used to assess the bioavailability of these pollutants. Subsequent analysis of this information along with biological data may be used to assess the impacts and risks of sediment pollutants to human populations, benthic populations/communities, and the ecosystem.

Monitoring of pollutant levels in sediments is a widely-accepted means of measuring the condition of the benthic habitat and is a powerful tool in the evaluation of spatial and temporal effects of anthropogenic and natural disturbance. However, for sediment chemistry data to be useful in interpreting toxicological response or in predicting environmental risks from sediment exposure, sediment criteria are needed. The Washington State Department of Ecology (1991) has adopted sediment criteria, and EPA has recently published a sediment compendium (USEPA, 1992). Both of these documents may provide useful guidance. The ability to extrapolate from sediment chemistry data to predictions of ecological or human health risks is, however, greatly limited by a limited understanding of causal relationships between exposure and response.

4.3.7 Summary and Recommendations

Rationale

- The sediments represent the ultimate sink for many chemical contaminants from offshore discharges.
- The objective of monitoring bulk sediment chemistry is to detect and describe spatial and temporal changes of these sediment pollutants.

Monitoring Design Considerations

- It is recommended that types of sampling gear and location and timing of sample collection be consistent to allow for comparisons among studies.
- Collection of undisturbed sediment requires that the sampler :
  - create a minimal bow wake when descending;
  - form a leakproof seal when the sediment sample is taken;
  - prevent winnowing and excessive sample disturbance when ascending; and
  - allow easy access to the sample surface in order that undisturbed subsamples may be taken.
- In general, analysis of the upper 2 cm is advised to examine recent sediment contamination events; however, site-specific conditions and program objectives should be considered when a sampling depth is selected.

- Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes.
- Total organic carbon and acid volatile sulfide normalizations are recommended to allow comparisons of chemical residue concentrations between locations. Normalizing the data will depend on the objectives of the individual monitoring program.
- It is recommended that sediments be sampled when contamination concentrations are expected to be at their highest levels.

Analytical Methods Considerations

- It is recommended that consistent analytical protocols be implemented to allow for comparisons between studies.
- Laboratories should perform acceptably on standard reference materials and intercalibration exercises before performing routine analyses.
- Periodic full-scan analyses should be undertaken to identify any new pollutants that should be monitored.
- Metals/Metalloids
  - The combination of GFAA and ICP is the recommended method for the detection of metals and metalloids.
  - Cold vapor AA is the recommended protocol for mercury detection.
- Organics
  - GC/MS in conjunction with isotope dilution is recommended for the detection of semivolatile and volatile organic compounds because it provides reliable recovery data for each analyte.
  - A vacuum super-cooled trap in conjunction with a purge-and-trap device is recommended for the detection of volatile organics.
- Sediment Toxicity Tests
  - May be necessary if the sediment contains contaminants that could result in an adverse impact on the benthic environment and/or the water column.
  - Results can be compared with reference-sediment test results to determine whether the site sediment has higher toxicity.

QA/QC Considerations

- The primary criterion for acceptable field sampling equipment and protocols is the collection of undisturbed and uncontaminated environmental samples.

- Adequate sampling containers and preservation techniques must be used and appropriate holding times followed to ensure the integrity of samples and their analyses.
- Blank, spike, and replicate analyses are recommended quality control checks.
- A report describing the objectives of the analytical effort; methods of sample collection, handling, and preservation; details of the analytical methods; problems encountered during the analytical process; any necessary modifications to the written procedures; and results of the QC analyses should be included with the analytical data.

Statistical Design Considerations

- Compositing of sediments consists of mixing two or more samples collected at a particular location and/or time period.
- Space-bulking (combining composites from several locations) and time-bulking (combining several composites over time from one location) should be used judiciously since information concerning spatial or temporal heterogeneity may be lost.
- Power analyses have shown that for a fixed level of sampling effort, a monitoring program's power is generally increased by collecting more replicates at fewer locations.

Use of Data

- The data gathered from monitoring of the pollutant levels in marine sediments should be used in conjunction with those collected from other physical and biological monitoring to monitor rates of recovery following environmental interventions, to evaluate the condition of benthic habitats, and to provide early warnings of potential impacts to the estuarine ecosystem.
- Sediment pollutant loading data should be used in combination with other monitoring methods to assess the condition of the benthic habitat or to guide the decision-making process.

4.4 SEDIMENT GRAIN SIZE

The objective of monitoring sediment grain size composition is to help describe spatial and temporal changes in the benthic community habitat. Grain size analysis measures the frequency distribution of particle size ranges composing the sediment. The size categories (in descending order) are gravel, sand, silt, and clay (Folk, 1980; Plumb, 1981). Sediment contaminants adsorb to small grain surfaces, and the availability of sediment contaminants may be correlated with the grain size composition of the benthic sediments.

4.4.1 Rationale

Of the 10 guidelines used to determine unreasonable degradation or irreparable harm, the following can be assessed by evaluating sediment grain size:

- Quantities, composition, and potential bioaccumulation or persistence of the pollutants to be discharged and
- Potential transport of the pollutants by biological, physical, or chemical processes.

Results from sediment chemical monitoring can be normalized according to sediment characteristics to account for some of the variability found in bioaccumulation rates. Also, grain size information may be used to aid in the analysis of the temporal and spatial variability of benthic assemblages since the ability of infaunal organisms to build tubes, burrow, capture food, and escape predation is affected by grain size. The results may be used to monitor rates of recovery following an event, to evaluate the condition of benthic habitats, and to assist in providing early warnings of potential impacts to the marine environment.

4.4.2 Monitoring Design Considerations

Sediment Sampling Devices

Sampling for sediment grain size is usually undertaken in conjunction with sediment chemistry and benthic infauna measurements. Thus, the sampling devices and techniques are similar for all three. In the past, sediment samples have been collected using a wide range of methods and samplers. Since sample collection protocols influence all subsequent laboratory and data analysis, it is essential that sediment samples be collected using acceptable and standardized techniques (USEPA, 1986-1991).

A variety of devices can be used to collect sediment samples, and these fall under two main categories: grab samplers and core samplers (McIntyre et al., 1984; ASTM, 1991). Each device samples the benthic habitat in a different manner (Table 4-18); therefore, comparisons among data collected using different devices is not recommended.

Table 4-18. Summary of Bottom Sampling Equipment[a]

Device	Use	Advantages	Disadvantages
Fluorocarbon plastic or glass tube	Shallow wadable waters or deep waters if SCUBA available. Soft or semiconsolidated deposits.	Preserves layering and permits historical sudy of sediment deposition. Rapid-samples immediately ready for laboratory shipment. Minimal risk of contamination.	Small sample size requires repetitive sampling.
Hand corer with removable fluorocarbon plastic or glass liners	Same as above except more consolidated sediments can be obtained.	Handles provide for greater ease of substrate penetration. Above advantages.	Careful handling necessary to prevent spillage, Requires removal of liners before repetitive sampling. Slight risk of metal contamination from barrel and core cutter.
Box corer	Same as above.	Collection of large undisturbed sample allowing for subsampling.	Hard to handle.
Gravity corers, that is, Phleger corer	Semiconsolidated sediments.	Low risk of undisturbed sample contamination. Maintains sediment integrity relatively well.	Careful handling necessary to avoid sediment spillage. Small sample, requires repetitive operation and removal of liners. Time-consuming.
Young grab (fluorocarbon plastic- or Kynar-lined modified 0.1 m^2 Van Veen)	Lakes and marine areas.	Eliminates metal contamination. Reduces pressure wave.	Expensive. Requires winch.
Ekman or box dredge	Soft to semisoft sediments. Can be used from boat, bridge, or pier in waters of various depths.	Obtains a larger sample than coring tubes. Can be subsampled through box lid.	Possible incomplete jaw closure and sample loss. Possible shock wave, which may disturb the fine sediments ("fines"). Metal construction may introduce contaminants. Possible loss of "fines" on retrieval.
Ponar grab sampler	Useful on sand, silt, or clay.	Most universal grab sampler. Adequate on most substrates. Large sample obtained intact, permitting subsampling.	Shock wave from descent may disturb "fines." Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.

Table 4-18. (continued)

Device	Use	Advantages	Disadvantages
BMH-53 piston corer	Waters of 4-6 ft depth when used with extension rod. Soft to semiconsolidated deposits.	Piston provides for greater sample retention.	Cores must be extruded on site to other containers. Metal barrels introduce risk of metal contamination.
Van Veen	Useful on sand, silt, or clay.	Adequate on most substrates. Large sample obtained intact, permitting subsampling.	Shock wave from descent may disturb "fines." Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.
BMH-60	Sampling moving waters from a fixed platform.	Streamlined configuration allows sampling where other devices could not achieve proper orientation.	Possible contamination from metal construction. Subsampling difficult. Not effective for sampling fine sediments.
Petersen grab sampler	Useful on most substrates.	Large sample; can penetrate most substrates.	Heavy, may require winch. No cover lid to permit subsampling. All other disadvantages of Ekman and Ponar.
Shipek grab sampler	Used primarily in marine waters and large inland lakes and reservoirs.	Sample bucket may be opened to permit subsampling. Retains fine-grained sediments effectively.	Possible contamination from metal construction. Heavy, may require winch. Tends to roll over sample.
Orange-peel grab Smith-McIntyre grab	Useful on most substrates.	Designed for sampling hard substrates.	Loss of "fines." Heavy, may require winch. Possible metal contamination.
Scoops, drag buckets	Various environments depending on depth and substrate.	Inexpensive, easy to handle.	Loss of "fines" on retrieval through water column.
Vibratory Corer	Used primarily for sandy unconsolidated sediments	Operational at all depths. Easily transported by truck and ship. Can penetrate deeper than conventional corers.	Has rarely been used for the study of environmental pollution.

[a]Comments represent subjective evaluations.
SOURCE: ASTM, 1991.

The collection of undisturbed sediment requires that the sampler:

- Create a minimal bow wake when descending;
- Form a leakproof seal when the sediment sample is taken;
- Prevent winnowing and excessive sample disturbance when ascending; and
- Allow easy access to the sample surface so undisturbed samples may be taken.

Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes. It is best to use a sampler with a weight adjustment to enable the modification of penetration depths.

Grab Samplers

Grabs are capable of consistent sampling of bottom habitats. Depending on the size of the device, areas from 0.02 to 0.5 m^2 and depths ranging from 5 to 15 cm may be sampled. Limitations of grab samplers include :

- Variability in penetration depth among samples depending on sediment properties;
- Oblique angles of penetration, which result in varying penetration depths within a sample; and
- Folding of the sample, which results in the inability to section the sample and the loss of information concerning the vertical structure in the sediments.

The careful use of these devices, however, will provide quantitative data. Grab samplers are the tools of choice for a number of marine monitoring programs (Fredette et al., 1989; USEPA, 1986-1991).

Core Samplers

Box corers use a surrounding frame to ensure vertical entry and, therefore, vertical sectioning of the sample (USEPA, 1986-1991). These devices are capable of maximum penetration depths of 15 cm and may collect volumes 5 to 10 times those of grab samplers. Limitations of box corers include :

- Large size and weight, which require the use of cranes or winches and a large vessel for deployment;
- Higher construction expenses; and
- Lack of calibration studies to permit comparisons to grab samples.

The Hessler-Sandia box corer uses dividers to section the core into subsections, facilitating subsampling of the core. Box corers are recognized as the tools of choice for maximum accuracy and precision when sampling soft-bottom habitats.

Sediment Profiling Camera

The sediment profiling camera allows vertical in situ imaging of the water-sediment interface, from which grain size determinations may be conducted. Sediment grain size determinations may be made at a maximum depth of 18 cm; however, penetration depth of the viewing prism may be limited because of the physical characteristics of the sediment (i.e., penetration depths are greater in silt than in sand).

Distinctions between finer silts and clays are not possible. Furthermore, it is recommended that quantitative calculations of sediment grain size from the image be ground-truthed (verified) by the results of laboratory analysis of collected sediment samples.

Although the singular use of the sediment profiling camera is not recommended, the camera may be effective as a reconnaissance tool. Delineation of habitats with similar physical characteristics may aid in the selection of appropriate sampling stations.

Recommendations

Collection of sediments and collection of benthic organisms should be done concurrently to mitigate the costs of field sampling and to permit sound correlation, regression, and multivariate analyses (see Section 4.5, Benthic Community Structure). Therefore, it is recommended that the sampling device also be suitable for benthic sampling. Grab and core sampling devices permit adequate sampling of both sediment and benthic infaunal communities with one sampling device.

Sample Depth

For characterizing surficial sediments, it is recommended that the upper 2 cm of the sediment column be evaluated; however, a minimum penetration depth of 4-5 cm should be achieved. Although the 2 cm specification is arbitrary, it does ensure that relatively recent sediments are sampled, that adequate volumes of sediment can be obtained using readily available samplers, and that data from different studies can be compared. Penetration depth is a function of sediment type and is greatest in fine sediments and least in coarse sediments. Sampling devices generally rely on either gravity or a piston mechanism to penetrate sediments. Ideally, the penetration depth can be varied by adding or subtracting weight from the sampler, but if the sampler cannot be made to consistently achieve the desired depth, an alternative device should be used (USEPA, 1986-1991).

Sampling of depths other than 2 cm or vertical stratification of deeper sediment cores may be appropriate, depending on the objectives of the monitoring program and the rate of sediment accumulation. For example, if information concerning only the most recent sedimentation events is required, examination of the upper 1 cm may be appropriate. Stratification of deeper cores will provide historical data of sediment grain size and depositional events. If the potential for bioaccumulation of contaminants in infaunal organisms is a concern, sampling to the depth of the anoxic layer is desirable. Comparison of data from studies analyzing different sediment depths is not advised. Sampler penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes.

Selection of Time of Sampling

Sediment grain size compositions are often temporally stable, although some slight seasonal variability may be present. Changes are usually associated with seasonal patterns of benthic turbulent mixing and sediment transport phenomena. The frequency of sampling should be related to the expected rate of change in grain size compositions. A consistent sampling period is recommended in order that spatial and temporal comparisons may be made.

If seasonal variations are exhibited, it is recommended that direct comparisons between samples collected during different seasons be avoided. Studies investigating interannual variation in the percent composition of grain sizes should be conducted during the same season (preferably the same month) each year. Furthermore, it is recommended that grain size be sampled under all discharge conditions so that changes in the sediment composition due to discharge loading can be tracked.

4.4.3 Analytical Methods Considerations

Sediment grain size may be expressed in either millimeters (mm) or phi (Φ) units. These scales are related according to the equation: $\Phi = -\log_2$ (mm). Data should be converted to phi units before calculation of grain size parameters. Sediments are broadly classified into three size classes: silts and clays are less than 0.064 mm (4 Φ) in diameter, sands range from 0.064 mm (4 Φ) to 1 mm (0 Φ) in diameter, and gravels are larger than 1 mm (Shepard, 1954; Folk, 1974). Grain size is normally reported as the mean (M_Z), although the median grain size (M_{d-}) is sometimes used. Sorting is a measure of the spread of the grain size distribution. Buller and McManus (1979) provide a good review of the methodological and statistical analysis of sediment samples.

Particle size determination can either include or exclude organic material. If organic material is removed prior to analysis, the "true" particle size distribution is determined. If organic material is included in the analysis, the "apparent" particle size distribution is ascertained. Most organic material is in the silt/clay size range and can be removed from the sediment either by acid washing or ashing. If organic material is left in the

sediments, it will tend to bias the results toward a smaller mean size. Because true and apparent distributions differ, detailed comparisons between samples analyzed by these different methods are questionable. It is therefore desirable that all samples within each study and among different studies be analyzed using one of these two methods, but all samples must be analyzed consistently. A standardized grain size analysis will allow all comparisons between samples within each study and between different studies.

Samples can be collected in glass or plastic containers. A minimum sample size of 100-150 grams is recommended. All samples should be stored at 4°C and can be held for up to 6 months before analysis. Samples cannot be frozen or dried prior to analysis since this may change the particle size distribution.

Before the sample is analyzed, it should be mechanically homogenized. The sample should be well-mixed during homogenization but should not be ground. If the organic fraction is to be removed, removal should be done by the addition of 10 percent hydrogen peroxide and subsequent boiling. The next step is to wet-sieve the sample to separate the sand and gravel fraction (greater than 62.5 μm) and the silt and clay fraction (less than 62.5 μm). The gravel-sand fraction can be subdivided by mechanically dry-sieving it through a graded series of screens. At minimum, the coarse subsample can be divided into the gravel fraction (greater than 2 mm) and the sand fraction (less than 2 mm) using a single filter with a 2-mm mesh size. A larger number of sieves can be used to subdivide these samples, with the number being determined by the needs of the monitoring program.

The silt-clay fraction is subdivided using a pipet technique that depends on the differential settling rates of particles of varying size. The sample is suspended in a 1-liter beaker of distilled water, and subsamples are removed at precise times (dependent on temperature) from a constant depth, as shown in Table 4-19 (USEPA, 1986-1991). The subsample is then dried and weighed to measure the mass of each size fraction. At a minimum, clay particles (less than 3.9 μm) should be separated from the silts (greater than 3.9 μm but less than 62.5 μm), but the number of fractions depends on the requirements of the monitoring program (USEPA, 1986-1991).

An alternative method for the determination of grain size for the sand fraction of the sediment is the use of a settling tube. This technique is based on Stoke's Law, which describes the sinking rate of a particle relative to its diameter. This technique requires a much smaller sample size (0.5-1.0 gm) as compared to the 100-gm sample required for dry sieving. Furthermore, settling tube analysis is relatively rapid, and automated settling tubes that input the data directly to a personal computer are available.

It is important that sieving techniques and the desired number of subfractions be specified and standardized to allow for comparisons between samples.

Table 4-19. Sediment Grain Size: Withdrawal Times for Pipet Analysis as a Function of Particle Size and Water Temperature[a]

Diameter Finer than (phi)	Diameter Finer than (μm)	Withdrawal Depth (cm)	Elapsed Time for Withdrawal of Sample, in Hours (h), Minutes (m), and Seconds (s)						
			18°C	19°C	20°C	21°C	22°C	23°C	24°C
4.0	62.5	20	20s	20s	20s	20s	20s	20s	20s
5.0	31.2	10	2m 0s	1m 57s	1m 54s	1m 51s	1m 49s	1m 46s	1m44s
6.0	15.6	10	8m 0s	7m 48s	7m 36s	7m 25s	7m 15s	7m 5s	6m55s
7.0	7.8	10	31m 59s	31m 11s	30m 26s	29m 41s	28m 59s	298m 18s	27m39s
8.0[b]	3.9	10	2h 8m	2h 5m	2h 2m	1h 59m	1h 56m	1h 53m	1h51m
9.0	1.95	10	8h 32m	8h 18m	8h 6m	7h 56m	7h 44m	7h 32m	7h22m
10.0	0.98	10	34 h6m	33h 16m	32h 28m	31h 40m	30h 56m	30h 12m	29h30m

[a] It is critical that temperature be held constant during the pipet analysis.
[b] Break point between silt and clay.
SOURCE: Modified from Plumb (1981).

4.4.4 QA/QC Considerations

It is critical that each sample be homogenized thoroughly in the laboratory before a subsample is taken for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field because sediments differentially sort themselves during transport and handling. In addition, after dry-sieving a sample all material must be removed from the sieve.

The total amount of fine-grained material used for pipet analysis should be 5-25 grams. If more material is used, particles may interfere with each other during settling and the possibility of flocculation may be enhanced; if less material is used, the experimental error in weighing becomes large relative to the sample size. Once the pipet analysis begins, the settling cylinders must not be disturbed because this will alter particle settling velocities.

It is recommended that triplicate analyses be conducted on 1 of every 20 samples, or on 1 sample per batch if fewer than 20 samples are analyzed. It is recommended that the analytical balance, drying oven, and temperature bath be inspected daily and calibrated at least once per week.

4.4.5 Statistical Design Considerations

Statistical strategies may mitigate the high costs of collecting sufficient quantities of sediment. Power analyses considering the strategy of compositing samples can often lead to a cost-effective monitoring design strategy.

Composite Sampling

Composite sediment sampling consists of mixing samples from two or more replications collected at a particular location at different times or at different locations at the same time. The analysis of a composite sample provides an estimate of an average contaminant concentration for the locations composing the composite sample. Advantages of the composite sampling strategy are:

- It provides a cost-effective strategy when individual analyses are expensive.
- It results in a more efficient estimate of the mean at specified sampling locations.

Because of the reduced sample variance, composite sampling results in a considerable increase in statistical power. If the primary objective of a monitoring program is to determine differences in grain size between sampling locations, composite sampling is an appropriate strategy.

Space-bulking consists of sampling from several locations and combining sediment samples into one or more composite samples. Time-bulking involves taking multiple samples over time from a single location and compositing these samples. Space- and time-bulking strategies should be used judiciously since significant information concerning spatial and temporal heterogeneity may be lost. The adoption of composite strategies will depend on the objectives of individual monitoring programs.

Statistical Power

Statistical power increases with an increase in the number of sediment subsamples in each replicate composite sample (USEPA, 1987d). However, with the addition of successive subsamples to each composite, a diminished return of statistical power exists. For composites of greater than 10 replications, the increase of power is negligible given typical levels of data variability.

To improve the power of a statistical test, while keeping the significance level constant, the sample size should be increased. Because of constraints in cost and time imposed by the monitoring program, however, this option may not be available. Power analyses have shown that for a fixed level of sampling effort, a monitoring program's power is generally increased by collecting more replicates at fewer locations. The number and distribution of sampling locations required to evaluate the effectiveness of the monitoring program will depend on the size and complexity of the discharge and the surrounding environment.

4.4.6 Use of Data

Results from sediment chemical monitoring can be normalized according to sediment characteristics to account for some of the variability found in bioaccumulation rates. Likewise, grain size information may explain the temporal and spatial variability in biological assemblages. These results may be used to monitor rates of change following the beginning of discharge, to evaluate the condition of benthic habitats, and to assist in providing early warnings of potential impacts on the marine ecosystem.

The singular use of sediment grain size to assess the condition of the benthic habitat or to guide the decision-making process is not recommended. The data collected from monitoring the physical characteristics of sediments should be used in conjunction with those collected from chemical and biological monitoring. Subsequent analyses of health risks to human populations and ecological risks to benthic individuals, populations, and communities may be performed. Sediment grain size provides evidence essential in the evaluation of spatial and temporal effects of ocean discharges.

4.4.7 Summary and Recommendations

Rationale

- The objective of monitoring the physical characteristics of sediments is to detect and describe spatial and temporal changes in sediment grain size.
- Results may be used to monitor rates of recovery following environmental interventions, to evaluate the condition of benthic habitats, and to assist in providing early warnings of discharge impacts to the surrounding ecosystem.

Monitoring Design Considerations

- It is recommended that the types of sampling gear and the location and timing of sample collection be consistent to allow for comparisons among studies.
- Collection of undisturbed sediment requires that the sampler :
  - create a minimal bow wake when descending;

  - form a leakproof seal when the sediment sample is taken;
  - prevent winnowing and excessive sample disturbance when ascending; and
  - allow easy access to the sample surface in order that undisturbed subsamples may be taken.

- Analysis of the upper 2 cm is advised in order to examine recent depositional events.

- Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes.

Analytical Methods Considerations

- Because true and apparent distributions differ, detailed comparisons between samples analyzed by different methods are questionable. It is therefore desirable that all samples within each study and among different studies in the study area be analyzed using only one method.

QA/QC Considerations

- Laboratory homogenization should be conducted even if samples were homogenized in the field.

- It is recommended that triplicate analyses be conducted on 1 of every 20 samples, or on 1 sample per batch if fewer than 20 samples are analyzed.

- It is recommended that the analytical balance, drying oven, and temperature bath be inspected daily and calibrated at least once per week.

Statistical Design Considerations

- Compositing sediment sampling consists of mixing samples from two or more replications collected at a particular location and time period.

- Space-bulking (combining composites from several locations) and time-bulking (combining several composites over time from one location) strategies should be used judiciously since information concerning spatial and temporal heterogeneity may be lost.

- Power analyses have shown that for a fixed level of sampling effort, the power of a monitoring program is generally increased by collecting more replicates at fewer locations.

Use of Data

- The singular use of sediment grain size monitoring to assess the condition of the benthic habitat or to guide the decision-making process is not recommended; data collected from monitoring the physical characteristics of sediments should be used in conjunction with those collected from chemical and biological monitoring.
- Results from sediment grain size monitoring can be used to normalize sediment pollutant results to account for some of the variability found in bioaccumulation rates.
- Grain size information may explain the temporal and spatial variability in biological assemblages.

4.5 BENTHIC COMMUNITY STRUCTURE

The objective of benthic monitoring is the detection and description of spatial and temporal changes in community structure and function. The results can assess the condition or "health" of benthic habitats and monitor the rates of recovery following environmental or human disturbances. Benthic fauna also represent a significant food source for many marine organisms, thereby providing an early warning of potential impacts on the marine ecosystem.

Benthic habitats may be broadly divided into hard- and soft-bottom substrates. Those habitats with soft-bottom substrates (e.g., sand, silt, mud) are composed of a significant benthic biological community known as infauna. This community consists of organisms that burrow into the substratum itself. The communities found over hard-bottom substrata such as coral reefs, rocky subtidal areas, and "live bottom" sponge/gorgonian habitats are referred to as epifauna. There are numerous species of polychaete worms, pelecypod molluscs, and sponges capable of penetrating the substratum; however, the majority of the community is found on top of the substratum. The latter hard-bottom or "live bottom" communities of dense sessile epifauna are valuable because they attract commercially and ecologically important species of fish (Darcy and Gutherz, 1984).

4.5.1 Rationale

Of the 10 guidelines used to determine unreasonable degradation and no irreparable harm, the following can be assessed by evaluating the benthic community structure:

- Composition and vulnerability of potentially exposed biological communities and
- Importance of the receiving water area to the surrounding biological community.

Monitoring the abundance of individual benthic organisms is a widely-accepted means of measuring the condition of the benthic habitat (Bilyard, 1987). Benthic organisms are exceptional indicators of benthic conditions since:

- They are usually sedentary; consequently, observed effects are in response to local environmental conditions, and their relative longevity of up to a year or more provides a level of consistency to data gathered.
- They are sensitive to habitat disturbance; communities undergo dramatic changes in species composition and abundance in response to environmental perturbations.
- They often mediate the transfer of nutrients and toxic substances in the ecosystem (via bioturbation and as important prey organisms).

Furthermore, monitoring benthic organisms is one of a handful of methods that provide in situ measures of biotic health and habitat quality. The assessment of benthic infaunal and epifaunal community structure is a powerful tool in the evaluation of spatial and temporal effects of anthropogenic and natural disturbance.

4.5.2 Monitoring Design Considerations

The level of effort required to assess benthic community structure is relatively high. A field survey is required to record and/or collect organisms, and sorting, identifying, and enumerating specimens require labor-intensive and generally expensive processes. Evaluations of benthic community structure and function may also be costly and time-consuming.

The results of benthic monitoring programs can vary substantially depending on the objectives and corresponding design specifications. The characteristics that are primarily responsible for the variability in the results are :

- Type of surveying and/or sampling gear;
- Sample sorting and/or data analysis protocols;
- Level of taxonomy; and
- Location and timing of sample collection.

The object is to standardize the measurement techniques at all stages of the 403 monitoring program to ensure that the data can be intercompared. Efforts should be made to try to match new monitoring data with historical data, but differences in techniques and sampling gear should be avoided. Historical data may be used only to note trends in the benthic fauna and, in particular, to follow any succession occurring as a result of increases in pollution.

Analyses of power-cost efficiencies are essential in selecting the appropriate survey technique and/or sampling gear and sample/data processing protocols. Ferraro et al. (1989) provide an example of power-cost analysis.

Different techniques are available for surveying and sampling organisms in hard- or soft-bottom habitats. Since these protocols will influence all subsequent laboratory and data analyses, it is essential that benthic samples be collected using acceptable and standardized techniques. Furthermore, samples of sediments and benthic organisms should be collected concurrently to mitigate the costs of field sampling and to permit sound correlation and multivariate analyses (see Sediment Chemistry). Therefore, it is recommended that soft-bottom sampling devices also be suitable for sampling the sediment (Table 4-18).

Hard-Bottom Habitat Surveys

In hard-bottom habitats, benthic surveys are used to document the relative cover of sessile organisms on the substratum and species distributions. Depending on weather and water conditions, these surveys can be performed by divers, or remotely operated vehicles (ROVs) or submarines can be used (Gamble, 1984; Holme, 1984). Specimens and sediment samples can then be directly collected by the diver or submarine to provide vouchers for positive taxa identification and for pathobiological or chemical analyses.

Types of Surveys

Numerous methods and techniques have been examined and compared to quantify hard-bottom community structure, particularly on coral reefs. These include simple line transects, quadrats, and plotless distance or nearest neighbor techniques. The data collected on species and organism coverage of the substratum allow calculations of species diversity, abundances, richness, and percent live tissue. Both randomly placed and "permanent" sites (marked by pins cemented into the substratum to allow accurate monitoring of the same site over time) have been used; each suffers from its own biases for statistical analyses. Each technique also presents problems in quantification and interpretation of data (see Loya, 1978; Gamble, 1984; Brown, 1988). Results of comparative studies indicate that:

- Although line transects are popular, they are not really very helpful unless multiple, closely-spaced 10-meter transects along same-depth contours are evaluated (Dustan and Halas, 1987).
- The linear point intercept method appears to be quite efficient and yields species abundance estimates similar to the true habitat (Ohlhorst et al., 1988).
- One-meter-square mapped quadrats provide good data for colony counts and estimates of percent coverage (Weinberg, 1981), but 4-meter-square quadrats are better for detecting rarer species.
- Photographed belt quadrats (1 x 10 meters-square) may provide the most information, although clear water conditions and long analysis time are necessary (Dodge et al., 1982).

Examples of line transects include a straight weighted line stretched over the substratum, with the species and lengths of live tissue underlying the line measured by tape, or the modification by Porter (1972), in which a chain of uniform links is placed over the corals and the number of links is counted and converted to area of live tissue cover. Rogers et al. (1983) used the ratio of the straight line to the length of chain required to cover the same distance as an estimate of the topographic relief of the substratum. Photogrammetry, in which still photographs are made of continuous areas under the transect line, provides a permanent record that can be quantified by computer

digitizing techniques (Bohnsack, 1979; Dodge et al., 1982; White and Porter, 1985). Further developments in this area include computer automation for analyses and using underwater video to record along transects. Although the latter technique is faster and easier to perform either by divers or ROVs, problems with quantifying the observations have not been worked out. However, video does provide an important visual record for assessing changes over time (Holme, 1984; Rogers, 1988).

For all of these methods, a number of trade-offs must be considered. Since most of this work must be performed by divers, diver safety is of the utmost importance, including the amount of bottom time available, the skill levels of the divers, and the type and degree of pollution that may be encountered. The types and amounts of information required for each species, as well as the length of time of the survey, will also affect the study design and techniques to be used. For many hard-bottom areas, there may not be one "best" method for monitoring and sampling the epifauna. Thus, two or more methods may be required to provide an adequate assessment of benthic community structure over time (see, for example, Dodge et al., 1982; Holme and McIntyre, 1984; Dayton, 1985; Witman, 1985; Rogers, 1988; Phillips et al., 1990).

Soft-Bottom Sampling Devices

In soft-bottom habitats, several types of devices can be used to collect benthic macroinvertebrate samples from the sediment: trawls, dredges, grabs, and box corers (McIntyre et al., 1984). The organisms collected in each sample are then identified and species distributions determined. Most of these devices sample the benthos in a unique manner. Accordingly, conducting comparisons between data collected using different devices is inadvisable. The same area and volume of sediment should be sampled since different species of benthic infaunal macroinvertebrates exhibit different horizontal and vertical distributions (Elliot, 1971).

Collection of an acceptable sediment sample for infaunal analyses requires that the sampler:

- Create a minimal bow wake when descending;
- Form a leakproof seal when the sediment sample is taken;
- Prevent winnowing and excessive sample disturbance when ascending; and
- Allow easy access to the sample surface in order that undisturbed subsamples may be taken.

Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes. It is optimal to use a sampler that has a means of weight adjustment to allow modification of penetration depths.

Trawls and Dredges

Trawls and dredges usually collect organisms over a variable and relatively large area (USEPA, 1986-1991; Fredette et al., 1989). The data collected using these devices are qualitative since consistent and reproducible sampling of a consistent benthic area is not possible. Their design and use preclude the collection of infaunal organisms at sediment depths greater than a few centimeters. Although trawls and dredges may be used to collect samples of either infaunal or epifaunal specimens for chemical/pathobiological studies, the specimens can be damaged by physical scrapes or breakage during this type of collection and adherent sand or silt particles could interfere with these analyses. However, these devices are suitable for reconnaissance of potential sampling sites.

Grab Samplers

Grab samplers are capable of consistent sampling of bottom habitats; depending on the size of the device, areas from 0.02 to 0.5 m^2 and depths ranging from 5 to 15 cm may be sampled. Limitations of grab samplers include:

- Variability among samples in penetration depth, depending on sediment properties;
- Oblique angles of penetration, which result in varying penetration depths within a sample; and
- Folding of the sample, which results in the inability to section the sample and the loss of information concerning the vertical structure in the sediments.

However, the careful use of these devices will provide quantitative data. Grab samplers are the tools of choice for a number of marine monitoring programs because of their ability to provide quantitative data at a relatively low cost. (Fredette et al., 1989; USEPA, 1986-1991).

Core Samplers

Core samplers use a surrounding frame to ensure vertical entry; vertical sectioning of the sample is possible (USEPA, 1986-1991). These devices are capable of maximum penetration depths of 15 cm and may collect volumes 5 to 10 times that of grab samplers. Limitations of box corers include :

- Large size and weight, which require the use of cranes or winches and a large vessel for deployment;
- Higher construction expenses; and
- Lack of calibration studies to permit comparisons to grab samples.

The Hessler-Sandia box corer uses dividers to section the core into subsections, facilitating subsampling of the core.

Box corers are recognized as the tools of choice for maximum accuracy and precision when sampling soft-bottom habitats. Smaller corers may also be handled by divers.

Suction Samplers

In situ suction devices use the flow of water to collect sediments and benthic organisms (Eleftheriou and Holme, 1984). An open-ended suction tube draws sediments and organisms into the flow of water, and the organisms are trapped in a sieve or mesh bag at the end of the tube. Limitations of suction devices include :

- Little control of the volume of sediment collected;
- Collection of animals from surrounding sediments, therefore inflating abundances; and
- Abrading organisms during collection, making identification even more difficult.

Fleishack et al. (1985) propose modifications to suction samplers that overcome some of these limitations. Although samplers can be operated remotely, most must be controlled by divers. Diver-operated collections are restricted to scuba depths and conditions that permit safe diving (e.g., relatively calm waters, reasonable visibility). This restriction limits their use in monitoring programs.

Sediment Profiling Camera

The sediment profiling camera is a unique tool that allows vertical, in situ imaging of the water-sediment interface. Photographs of biological activity may be made at a maximum depth of 18 cm; however, the penetration depth of the viewing prism may be limited because of the physical characteristics of the sediment (i.e., penetration depths are greater in silt than in sand).

The sediment profiling camera system can give measurements of organism tube density, thickness of the pelletal layer, and infauna present in the image area (Rhoads and Germano, 1982). Although this tool provides qualitative information concerning benthos activity, the sediment profiling camera system cannot provide quantitative information on species diversity, abundance, and biomass. Information concerning species composition cannot be gained using this tool. Consequently, characterizations of the benthic community structure cannot be made from the data taken from these images.

The sediment profiling camera is not recommended for the collection of benthic community structure data; however, it is an effective reconnaissance tool. Delineation of physically similar sampling sites may be determined through the use of this tool, aiding in the selection of sampling stations. Ground-truthing of these images by means of laboratory analyses of collected material is highly recommended.

If quantitative analyses of infaunal benthic community structure are required, a grab or core sampler is recommended. Either of these sampling devices permits adequate sampling of both sediment and benthic infaunal communities with one sampling device.

Sampling Area

The numerous species of benthic macroinvertebrates have varying scales of spatial distribution both horizontally and vertically (Elliot, 1971; Livingston et at., 1976; Loya, 1978; Downing, 1979). Field and laboratory costs increase with increasing sample size; therefore, the determination of the optimal sample size must consider spatial and temporal scales, as well as statistical power. Preliminary community surveys should be undertaken to determine the most appropriate technique for the site(s) and the optimal balance between the types of information and/or samples needed and the time available for each survey/sampling session and subsequent analyses. In particular, the limitations and requirements of the biological indices used in the data analyses to determine community structure must be carefully considered to set the optimal sampling area.

Selection of Sampling Period

Benthic infauna assemblages are dynamic; the most common temporal patterns observed in benthic communities are those associated with seasonal changes. Seasonal variation in benthic assemblages may be due to changes in physical, chemical, and/or biological parameters, e.g., temperature, light transmissivity, dissolved oxygen, predation, recruitment, etc.

Given the seasonal variation characteristic of benthic assemblages in general, it is recommended that direct comparisons between samples collected during different seasons be avoided. Studies investigating interannual variation in the characteristics of benthic communities should be conducted during the same season (preferably the same month) each year.

Data Collection Protocols

For both hard and soft bottoms, the species present and numbers of individuals need to be determined to characterize the benthic community structure. During hard-bottom surveys, this information may be collected by the diver (recording the data on an underwater slate or waterproof paper, on which the format for either transect or quadrat information has been preprinted to facilitate data recording), or it may be derived from photographs (examined by light microscopy or computer scanning techniques) at a later

date. The organisms collected by grab or corer devices from soft bottoms must be rinsed from the sediments, preserved, and sorted for identification and enumeration. Several options must be considered when designing sorting protocols. To ensure comparability between studies, a consistent set of sorting procedures is highly recommended.

Sieving: Mesh Size and Location

The use of different sieve mesh sizes limits the comparability of results between marine monitoring studies (Reish, 1959; Rees, 1984). The major advantage of using a smaller mesh size is the retention of both juvenile and adult organisms as well as large- and small-bodied taxa. The major disadvantage is the concomitant increased cost of sample processing. For example, using a 0.5-mm mesh over a 1.00-mm mesh could increase retention of total microfaunal organisms by 130-180 percent; however, costs for processing the samples may increase as much as 200 percent (USEPA, 1986-1991).

It is recommended that a standard mesh size be selected for all monitoring studies. The 1.0-mm mesh size is commonly used in impact assessments, and the 0.5-mm mesh size is sometimes used in recruitment studies. The issue of data comparability was resolved by recommending sequential screening and analysis procedures (Becker and Armstrong, 1988). By screening samples through both the 1.0- and 0.5-mm sieves, the 1.0-mm fraction could be analyzed separately and the data compared with other impact studies. The data for the 0.5-mm fraction of the samples could then be combined to yield information on the complete sample for comparison with other recruitment data.

Sieving can be done either aboard the survey vessel or on shore after the cruise. Sieving occurs prior to fixation (sample preservation) aboard the vessel, whereas waiting until after the cruise requires fixation prior to sieving. If inadequate concentrations of fixative are added and deterioration or decomposition of organisms occurs, there may be a significant detrimental modification of the sample. If large numbers of samples are to be collected, field sieving reduces the sample volume required for storage, as well as the modification/loss of data.

Use of Relaxants

The use of relaxants prior to sieving and fixation reduces the tendency of organisms to distort their shape or autotomize, and consequently facilitates taxonomic identifications and morphometric measurements (USEPA, 1986-1991). Complete organisms, having a "natural" appearance, are easier to identify to a lower taxonomic group than are distorted or fragmented specimens.

The magnitude to which relaxants can influence taxonomic identification, thereby limiting comparisons between monitoring studies, is unknown. It is not recommended that comparisons be conducted between studies in which relaxants were used and those in which they were not used.

Use of Stains

Vital stains—primarily rose bengal—are added to samples to facilitate sorting. The stain colors most infauna, enhancing their contrast with background debris. Not all taxa stain adequately, and some taxonomists have found that staining interferes with the identification of certain taxa. Although staining may aid the sorting process, a proper quality control program should ensure that sorting efficiency is maintained whether or not a stain is used.

Level of Taxonomy

The necessity to identify organisms to the level of species has recently been questioned (Warwick, 1986). The liabilities of identifying organisms to the species level include :

- Identifications may be inaccurate and imprecise (Ellis, 1985).
- Identifications are time-consuming and costly; costs can be reduced as much as 30-50 percent by limiting identification of samples to higher taxonomic groups (USEPA, 1986-1991).

Identifications to higher taxonomic groups can provide gross characterizations of benthic assemblages and may be sufficient to meet program objectives concerning detection of community responses to anthropogenic disturbance. Warwick (1986) contends that detection of anthropogenic disturbances would be facilitated since these perturbations affect community structure at taxonomic levels higher than the species level. Natural disturbances, which generally affect community structure by species replacement, would have little influence on analyses at higher taxonomic levels.

However, identification to higher taxonomic levels may sacrifice the potential wealth of information available using species-level identification such as analyses of population dynamics and productivity. Furthermore, it is generally regarded that species is the taxon most susceptible to environmental stress; individuals tend to be replaced by another species within the same genus before the genus is replaced (USEPA, 1986-1991).

The level of taxonomic identification will depend on monitoring program objectives, sample size, study sites, analytical measure, and availability of trained taxonomists. Data based on lower taxonomic levels can be grouped for future comparisons with higher level taxa. It is strongly recommended that all samples identified only to higher taxonomic levels be properly archived, since comparisons of lower taxonomic data may

be required later. For in situ hard-bottom surveys, divers must have adequate training to recognize the species being examined. When in doubt, or for species that are difficult to identify in the field (e.g., soft corals, sponges), voucher specimens must be collected, properly labeled, and submitted to specialists for identification.

4.5.3 Analytical Methods Considerations

A variety of approaches are available to assess the effects of discharges on benthic soft- or hard-bottom communities. Many assessment approaches are derived from modifications of Connell's (1978) Intermediate Disturbance Hypothesis. Some researchers have proposed that selected community structure measures (e.g., species composition, abundance) are predictable along a gradient of environmental disturbance (Pearson and Rosenberg, 1978; Gray and Mirza, 1979; Warwick, 1986). A corollary to this supposition is that indicator species whose responses would epitomize community responses to habitat perturbations can be identified.

These assessment approaches may be grouped into three categories:

- Biological indices,
- Indicator species, and
- Multivariate analyses.

However, there has been little consensus among biologists regarding the suitability of various techniques for describing community characteristics and/or for assessing impacts due to discharges. A critical evaluation of the use of biological indices to detect environmental change is presented in USEPA, 1985d (Table 4-20). In addition to measures of change in the abundances of pollution-sensitive, pollution-tolerant, and opportunistic species, the indices shown in Table 4-20 are evaluated on the basis of the following criteria:

- Biological meaning,
- Ease of interpretation, and
- Sensitivity to community changes due to anthropogenic sources.

The results of these evaluations and additional information on other analytical methods are summarized below.

Biological Indices

The numbers of individuals and the numbers of species have been found to be good indicators of anthropogenic disturbance, as well as of other environmental stresses (Table 4-20; Pearson and Rosenberg, 1978; Warwick, 1985). Furthermore, these simple biological indices are less ambiguous and are often as informative as diversity

Table 4-20. Biological Indices

Index/Method	Biological Characteristic Measured	Recommended for Monitoring[a]
Biological integrity	Community structure	B
Bray-Curtis	Dissimilarity	P,B
Dominance[b]	Community structure	P,B
Infaunal index	Community structure	B[c]
Number of individuals	Total abundance	P,B
Number of species	Total taxa	P,B
Opportunistic and pollution-tolerant species	Community structure	P,B
Pollution-sensitive species	Community structure	P,B
Cover	Living tissue	B
Biomass	Standing crop	P,B
Margalef's SR	Diversity	P,B
J	Evenness	P,B
Shannon-Weiner H′	Diversity	P,B

[a]P(plankton), and B(benthos) indicate those biological groups to which a given index may be applied.

[b]Defined as the minimum number of species required to account for 75 percent of the individuals in a sample (see Swartz et al., 1985).

[c]Where developed.

indices (USEPA, 1985d; Green, 1979; Hurlbert, 1971). Measures of biomass have inherent problems in the collection of the data, e.g., loss or gain of weight due to preservative medium, drying times, and evaporative weight loss. However, biomass, used in conjunction with species composition and abundance data, may demonstrate the relative effects of enrichment on benthic communities (Pearson and Rosenberg, 1978).

More complicated indices—e.g., species diversity, species richness, dominance, evenness—have found varying degrees of acceptance. Diversity indices, measures of the distribution of individuals among species, have the following limitations (Green, 1979):

- Often lack biological meaning;
- Are not robust empirical indicators of ecosystem "health";
- Do not incorporate information on form and function of resident species; and
- Are susceptible to biases associated with well-described taxa.

However, species diversity indices are a widely used measure of community structure and provide a consistent level of data reduction for large-scale comparisons.

The dominance index is a measure of the degree to which one or a few species dominate the community. The dominance index, herein defined as the minimum number of species required to account for 75 percent of the total number of individuals, has been useful in describing community structure (Swartz et al., 1985). It is easily calculated, does not assume an underlying distribution of individuals among species, and is statistically testable.

Several graphical analyses of these simple biological indices have been developed although most require further study before their effectiveness is known. For example, the use of Gray and Mirza's (1979) lognormal distribution of individuals per species and Warwick's (1985) abundance-biomass comparison (ABC) methods, though useful for some habitats, have been found to be unpredictable for other habitats and other locales (USEPA, 1989e; Beukema, 1988). However, Pearson and Rosenberg's (1978) species-abundance-biomass (SAB) model appears to be applicable to several forms of environmental disturbance and may be used in conjunction with other analyses to assess spatial and temporal impacts due to disturbance (Figure 4-2). The SAB model provides a means to assess the condition of the habitat based on species numbers, total abundance, and total biomass. Severely polluted areas are identified by the lack of organisms, low species numbers, total abundance, and biomass. Highly polluted areas are identified by a few, highly abundant populations of small-bodied, pollution-tolerant, opportunistic species (e.g., *Capitella capitata*); total abundance is high, although biomass and species numbers remain low. Less polluted areas (e.g., farther from a

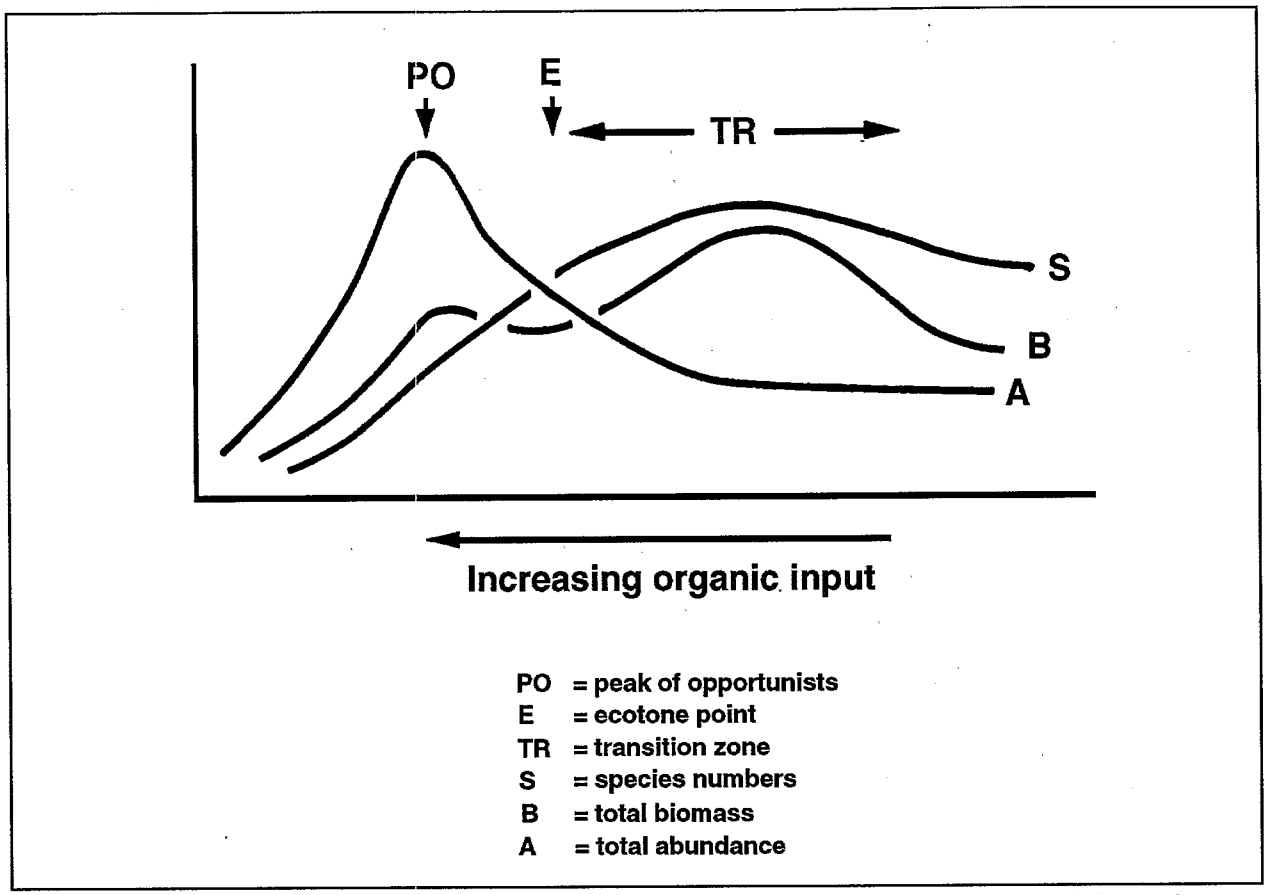


Figure 4-2. Generalized SAB Diagram of Changes Along a Gradient of Organic Enrichment (from Pearson and Rosenberg, 1978)

point source, or over time following the cessation of organic input) may be identified by high species numbers and biomass, as more competitive, larger-bodied species are able to tolerate sediment conditions. More pristine areas are characterized by a number of small populations of competitively dominant species; species numbers remain elevated, although biomass and total abundance are low. This approach, however, requires the comparison of two or more sites along the pollution gradient.

For hard-bottom communities, some of the methods used successfully for detection of pollutant impacts in soft-bottom communities (Warwick, 1986) appear to have little direct application (Brown, 1988). For example, the sensitivity of diversity indices to describe change in coral communities may be less than that of other parameters such as coral cover, colony size, and abundance, suggesting that each parameter requires careful analysis based on the previous environmental history of the site, the nature of the impact, and the community structure of the reef. Grigg and Dollar (1990) have proposed a stress index that measures net mortality of the organisms over time, partitioning the mortality due to natural causes (N) and that due to man-made influence (I), thus allowing these contributions to be compared. In terms of survival then:

$$\text{Stress Index} = 1.0 - (N + I),$$

where N + I varies from 1 (total destruction) to 0 (zero mortality).

Although there are problems in applying this index in the field, development of a quantitative index of stress would assist in the assessment of natural versus anthropogenic contributions in hard-bottom benthic communities (Brown, 1988).

Indicator Species

Examination of abundances of individual indicator species are generally informative and may reduce the cost of the analysis. The absence of pollution-sensitive species and the enhancement of opportunistic and pollution-tolerant species may assist in defining the spatial and temporal extent and magnitude of impacts. However, indicator variables must possess the following characteristics (Green, 1979):

- Must provide a sufficiently precise and accurate appraisal of:
  - species of concern,
  - anthropogenic disturbances to benthic communities, and
  - presence/absence or magnitude of anthropogenic perturbation to the ecosystem.
- Must provide a cost-effective and statistically reliable alternative to monitoring all critical benthic community measures of habitat perturbation.
- Must be appropriate for the spatial and temporal scale demanded by the study objectives.

Pearson and Rosenberg (1978) demonstrated that along a gradient of organic enrichment, a predictable community of benthic infauna could be observed (Figure 4-3). Communities consist of opportunistic, tolerant species in areas of severe pollution, giving way to less tolerant and more competitively dominant species further from severely polluted areas (Figure 4-3). This model of indicator species composition and distribution has been found to be useful in assessing both natural and anthropogenic disturbances.

Several indices, derived from the distributions of pollution-sensitive species and opportunistic disturbance-tolerant species, have been developed to evaluate impacts to infaunal benthic community structures: copepod/nematode ratio (Raffaelli and Mason, 1981), infaunal trophic index (Word, 1978), and organism-sediment index (Rhoads and Germano, 1986). The infaunal index, which was initially developed for the Southern California Bight, uses the abundances of four functional groupings to describe community structure. Low values of the infaunal index indicate communities dominated

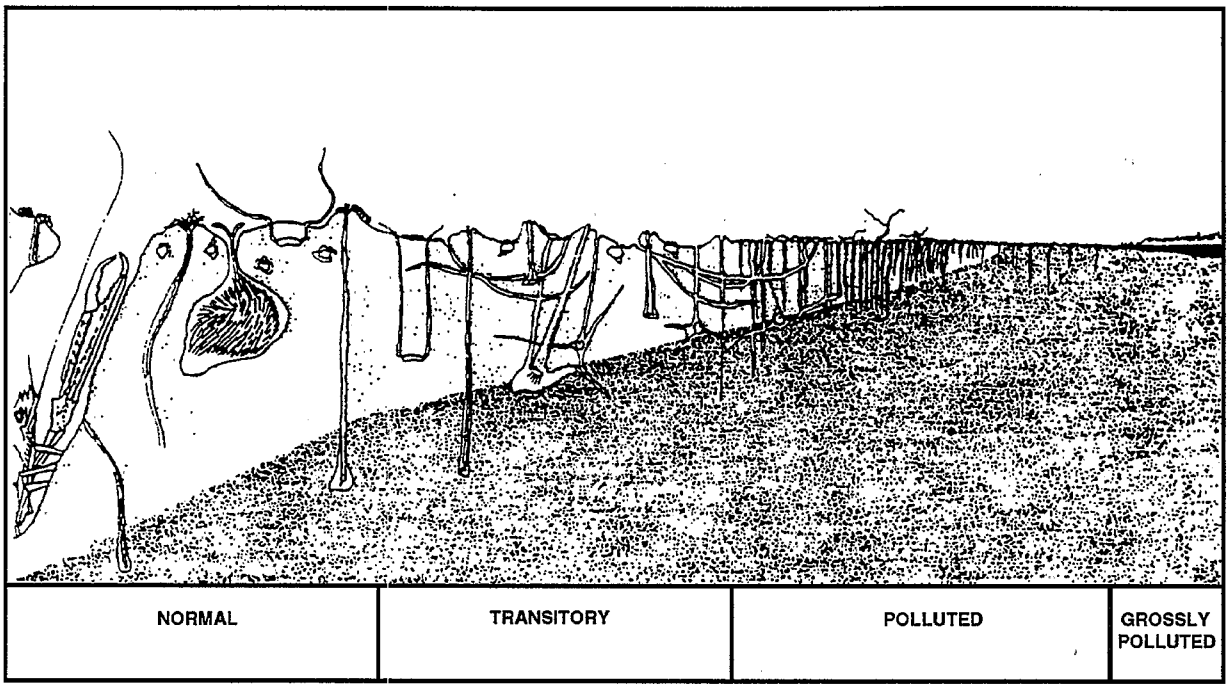


Figure 4-3. Diagram of Changes in Fauna and Sediment Structure Along a Gradient of Organic Enrichment (from Pearson and Rosenberg, 1978)

by deposit-feeding organisms that are considered to be more impacted than communities dominated by suspension feeders (high infaunal index values)(Word, 1978).

Polluted or disturbed conditions may be indicated by:

- High rates of the nematode/copepod ratio;
- Low values (A) of the infaunal index; and
- Low values (7) of the organism-sediment index.

However, further studies of the response patterns of infaunal species subjected to anthropogenic perturbations are required in order to select appropriate indicators of benthic community impact. Again for hard-bottom communities, the use of epifaunal indicator species and other indices requires further research (Brown, 1988).

It is recommended that multiple pollution-tolerant and pollution-sensitive indicator species be selected by clearly defined criteria. Multiple indicator species often provide a more complete representation of environmental conditions.

Multivariate Analyses

Numerical classification encompasses a wide variety of techniques that have been used in the analysis of benthic data to distinguish groups of entities (e.g., sample locations) according to similarity of attributes (e.g., species). These techniques differ from most multivariate methods in that no assumptions are made concerning the underlying distributions of the variables. Detailed descriptions of numerical classification analysis can be found in Pielou (1984), Romesburg (1984), Clifford and Stephenson (1975), Boesch (1977), Sneath and Sokal (1973), and Anderberg (1973). Boesch (1977) is particularly valuable as an introduction and guide to the use of numerical classification analysis in marine environmental studies. Guidance on the interpretation of classification results is provided in an EPA Technical Support Document (USEPA, 1988d).

Ordination analyses have also been used to reduce the dimensionality of the data set while maintaining the relationship among similar and dissimilar entities. At present, no single ordination technique has been shown to be clearly superior for the analysis of biological data (USEPA, 1985d).

Multivariate analyses are effective heuristic tools. They generate visual representations that often indicate where further analyses ought to be conducted.

Analytical Approach Recommendations

Some of the most informative measures of community structure are the simplest (Table 4-20):

- Number of individuals,
- Number of species,
- Dominance,
- Infaunal index,
- Abundances of pollution-sensitive species, and
- Abundance of opportunistic and pollution-tolerant species.

These indices have proved to be useful over various habitats and regions in assessing changes to benthic community structures (USEPA, 1985d). Values of these indices may be determined from the list of species abundances generated during the taxonomic identifications of collected specimens. Furthermore, the values of these six variables may be easily tested statistically using parametric or nonparametric techniques. It is recommended that no single index or analytical method be used to assess impacts; instead, the assessment of impacts should incorporate information that each variable and method contributes concerning benthic community structure.

Selection of reference sites is key to the evaluation of environmental impact assessment. Results of analyses using reference measures provide the means of comparison by which anthropogenic impacts are detected. It is essential that selected reference sites exhibit at least similar:

- Sediment characteristics (i.e., grain size or substratum type),
- Water depths,
- Flow characteristics,
- Salinity,
- Dissolved oxygen, and
- Temperature

compared to monitoring program sampling sites. Several reference sites may be required to meet these criteria.

4.5.4 QA/QC Considerations

Sample Collection

Surveying and sampling equipment should be inspected for wear and tear to avoid loss of data or sample leakage and loss upon ascent. It is recommended that backup survey and sampling equipment be available on board the vessel in case the primary equipment breaks down or is lost during the cruise.

The following infauna sample acceptability criteria should be satisfied (USEPA, 1986-1991):

- Sediment is not extruded from the upper face of the sampler such that organisms may have been lost.
- Overlying water is present, indicating minimal leakage.
- The sediment surface is relatively flat, indicating minimal disturbance or winnowing (Figure 4-4).
- The entire surface of the sample is included in the sampler.
- The desired penetration depth is achieved.

If the sample does not meet all the criteria, it should be rejected.

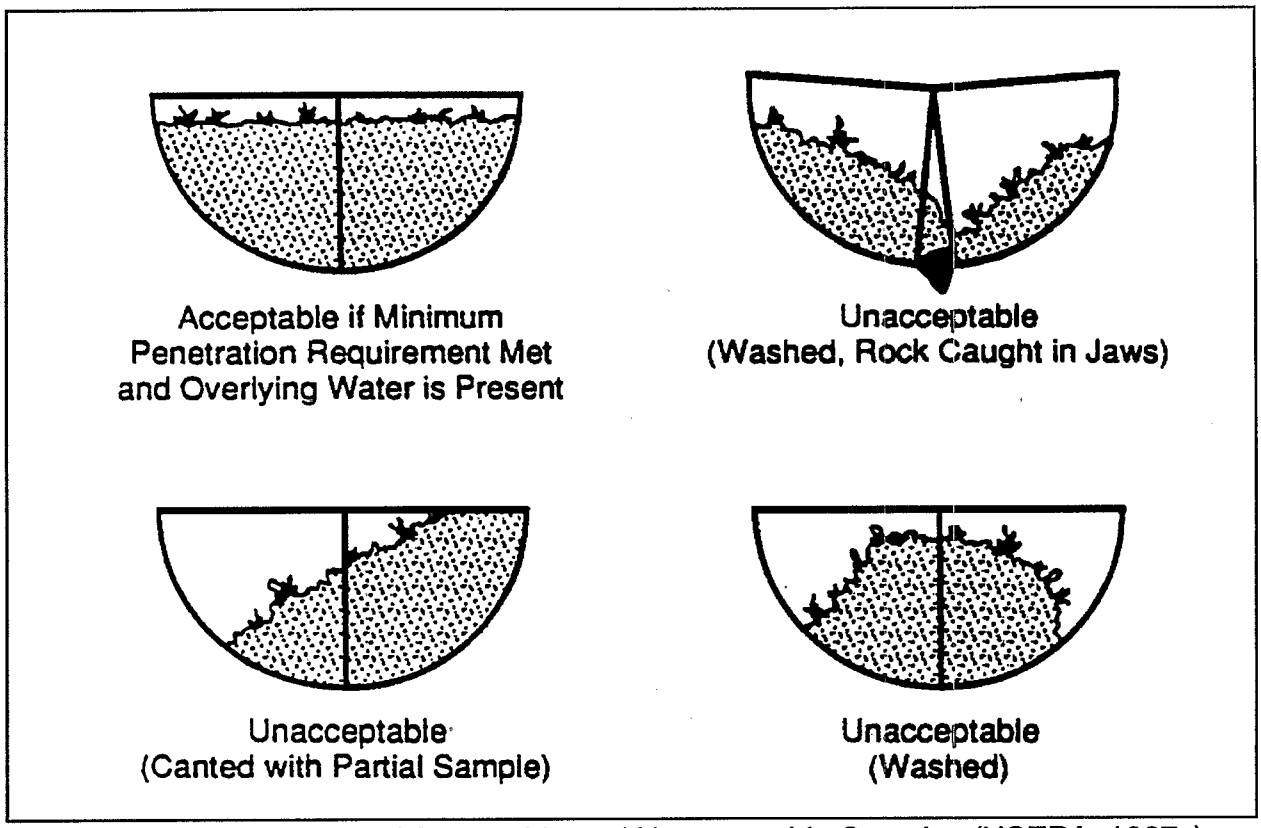


Figure 4-4. Examples of Acceptable and Unacceptable Samples (USEPA, 1987c)

Appropriate QA/QC protocols for assessing the validity of hard-bottom surveys (e.g., multiple transects, resurveying one or more transects or quadrats by different divers) should also be followed.

Taxonomic Identification

A key QA/QC issue is taxonomic standardization. Consistent taxonomic identifications are achieved through interaction among taxonomists working on each major group. Participation of the laboratory staff in regional taxonomic standardization programs is recommended to ensure regional consistency and accuracy of identifications.

Five percent of all samples identified by one taxonomist should be reidentified by another taxonomist who is also qualified to identify organisms in that major group.

It is advisable that at least three individuals of each taxon should be sent for verification to recognized experts. These verified specimens should then be placed in a permanent reference collection. All specimens in the reference collection should be stored in labeled vials that are segregated by species and sample. Reference specimens should be archived alphabetically within major taxonomic groups.

It is also recommended that at least 20 percent of each sample be re-sorted for QA/QC purposes. Re-sorting is the examination of a sample or subsample that has been sorted once and is considered free of organisms. Re-sorting should be done by someone other than the one who sorted the original sample. If a sample is found that does not meet the recommended 95 percent removal criterion, the entire sample should be re-sorted. For epifaunal surveys, a portion of the photographed quadrats should be reanalyzed for comparisons.

4.5.5 Statistical Design Considerations

Consideration of statistical strategies will mitigate the high costs of collecting and processing samples.

Temporal Stratification of the Data

The time of the year should be controlled or stratified in the design; the use of annual averages is seldom a good practice. Temporal stratification of the data should not be attempted until sufficient knowledge of long-term natural cycles is attained. Initially, simple regression analyses may be conducted on seasonally stratified data to identify monotonic temporal trends. Further examinations of whether conditions are improving or degrading over time may be performed using various statistical time series analyses.

Statistical Power

Selection of the number of replicates is an important component of program design. The inherent patchiness of benthic communities requires collection of sufficient replicate samples to ensure an accurate description of the benthos. However, increases in replication increase sample processing costs. Power analyses assist in the allocation of sampling resources (stations, replication, and frequency) with regard to program finances and design.

Power analyses may be applied to determine the appropriate number of sample replicates required to detect a specified difference (USEPA, 1987d). The number of replications required to detect a specified minimum difference is a function of the statistical power and the variance in the data. Power analyses require a priori knowledge of the variability in the data. A best guess or, preferably, variation observed in historical data is often used initially in the design of the monitoring program.

To improve the power of a statistical test, while keeping the significance level constant, the sample size (area sampled, number of replicates of grabs, quadrats, or transects) should be increased. Because of constraints in cost and time, however, this option may not be available. Power analysis has shown that for a fixed level of sampling effort, a monitoring program's power is generally increased by collecting more replicates at fewer locations. Sampling should be conducted in a radiating pattern from the zone of initial discharge out to the distance where there will be no effects.

4.5.6 Use of Data

Monitoring of benthic community structure provides in situ measures of the benthic habitat and is a powerful tool in the evaluation of spatial and temporal effects of anthropogenic and natural disturbance. The presence or absence of certain organisms is useful in indicating the previous condition of the environment (Bilyard, 1987; Tomascik and Saunder, 1987). Monitoring of benthic infaunal communities also provides data required in the design and validation of benthic community dynamics models (Pearson and Rosenberg, 1978; Brown, 1988) and the selection of biological indicators (Word, 1978).

In addition, monitoring of benthic communities directly provides accurate information essential in assessing the effectiveness of discharge reductions (Bilyard, 1987). For example, benthic infauna monitoring provided information used to assess the effectiveness of pollution abatement plans in the recovery of Southern California waters (Reish, 1986; SCCWRP, 1988). These studies indicate that analyses of benthic communities may be effectively used to monitor the long-term health of the receiving environment (Reish, 1986).

Currently, benthic communities have not proved useful for identifying specific chemicals or classes of chemicals present in the environment. Further information concerning specific responses to specific contaminants is required before infaunal community structure becomes useful in identifying specific contaminants (USEPA, 1989e). In addition, caution is recommended in the use of benthic community structure to predict specific effects on potential predators. Information on trophic relationships, competition, and predation is often not available. The capability to predict the effects of altered prey communities on predators may improve with research on these topics. Factors such as prey quality, distribution of prey, and interactions among species will be important components of this research.

However, benthic invertebrates do serve as effective indicators of environmental condition, delineating the magnitude, spatial extent, and temporal trends of anthropogenic and natural perturbations to the ecosystem (Reish, 1986; Bilyard, 1987). Monitoring of benthic infauna and epifauna will provide relevant accurate data fundamental to achieving the objectives of most monitoring programs.

4.5.7 Summary and Recommendations

<u>Rationale</u>

- The objective is to detect and describe spatial and temporal changes in the structure and function of benthic communities.

- Benthic monitoring provides in situ measures of habitat quality and is a powerful tool in assessing environmental impact.

Monitoring Design Considerations

- It is recommended that consistent types of surveying and sampling gear, data collection and sample sorting protocols, level of taxonomy, and location and timing of sample collection be implemented to allow for comparisons between studies.
- To reduce the variation due to seasonal differences, sampling should be conducted during the same season—preferably the same month—each year.
- Voucher specimens should be collected for in situ epifaunal surveys.
- For epifaunal surveys, data are collected on species and organism coverage of the substratum by diver, ROV, or submarine from specified transects or quadrats.
- For infaunal sampling, collection of undisturbed sediment requires that the sampler :
  - create a minimal bow wake when descending;
  - form a leakproof seal when the sediment sample is taken;
  - prevent winnowing and excessive sample disturbance when ascending; and
  - allow easy access to the sample surface in order that undisturbed subsamples may be taken.
- Penetration well below the desired sampling depth is preferred to prevent soft-bottom disturbance as the device closes.
- Grab samplers and box corers are recognized as the tools of choice for maximum accuracy and precision when sampling soft-bottom habitats.
- Sorting through a standard sieve mesh size (i.e., 0.5 mm) is recommended. Further sorting through other mesh sizes may be conducted in addition to sorting through this standard mesh size.
- Relaxants facilitate identification and morphometric measurements; however, standard procedures must be implemented to ensure valid comparisons among studies.
- Vital stains may facilitate sorting; however, a proper QA program should ensure that sorting efficiency is maintained.
- Identifications to higher taxonomic levels may be sufficient to meet program objectives; however, it is recommended that all samples be archived if comparisons to lower taxonomic levels will be required at a later date.

Analytical Methods Considerations

- It is recommended that simple measures of community structure be used to assess the condition of the benthos: number of individuals, number of species, dominance, infaunal index, abundance of pollution-sensitive species, abundance of pollution-tolerant species, and percent cover for hard-bottom indicator groups.
- Selected biological indices should retain biological meaning, be robust indicators of ecosystem "health," and incorporate species form and function.
- Indicator species should possess the following characteristics:
  - sensitive to benthic perturbations of concern;
  - cost-effective and statistically reliable alternative to measuring all species in a monitoring program;
  - statistically reliable indicative measures of habitat perturbations; and
  - appropriate for the spatial and temporal scale demanded by the study objectives.
- Selection of reference sites is key to the evaluation of environmental impact due to anthropogenic impacts; several reference sites may be required.

QA/QC Considerations

- Taxonomic standardization is essential to the analysis of community structure. Recommended protocols include consistent interactions among taxonomists, reidentification of selected samples, use of a reference collection, and re-sorting and analysis of selected samples or subsamples.

Statistical Design Considerations

- Power analyses may be applied to determine the appropriate number of sample replicates required to detect a specified difference, thereby optimizing the high costs of collecting and processing samples.

Use of Data

- Data provide essential information in order to assess impacts due to anthropogenic perturbation, monitor recovery of the receiving environment, and validate community and population models.

4.6 FISH AND SHELLFISH PATHOBIOLOGY

Pathobiological methods provide information concerning damage to organ systems of fish and shellfish through an evaluation of their structure, activity, and function. Anatomic pathology methods can give an indication of the nature of an altered state, for example, by identifying the specific type of tumor present in an animal. Reproductive developmental studies examine the reproductive capacity of animals and can provide information to aid in estimating and predicting population abundance and recruitment. Biochemical/enzymological studies seek to detect differences in enzymatic activity as a measure of biological condition. Immunological methods can demonstrate altered immune response, an indicator of changes in bodily defense mechanisms and increased susceptibility to disease.

Pathobiological methods should be used in concert to investigate cause-and-effect relationships as a result of contaminant exposure. Anatomic pathology can serve as a vital link between observed effects on populations and communities in an estuary and the changes in activity and function observed by other methods.

4.6.1 Rationale

Pathobiological methods can be used to examine adverse effects of pollutants on fish and shellfish. The presence of toxics in water and sediments may not immediately result in visible changes in these organisms. Biomarkers offer a more sensitive and reliable assessment of exposure risks than ambient water or sediment quality monitoring. Monitoring of pathobiological effects provides information necessary to make determinations of the existence of adverse effects in animals (e.g., tumors), population productivity and stability (affected by reproduction and disease states), and the loss of organisms deemed valuable for ecological, aesthetic, recreational, scientific, or economic reasons. Table 4-21 outlines some of the terms used to describe pathobiological methods.

Although the value of these methods for establishing cause-and-effect links has been established during laboratory toxicity studies, some questions remain regarding their ability to establish such links for field-collected organisms that are exposed to a variety of natural environmental stresses and combinations of contaminants (Hinton and Couch, 1984; Couch and Harshbarger, 1985; Mix, 1986; Sindermann, 1990). However, properly conducted multidisciplinary monitoring studies using these methods can provide regulatory agencies with evidence of impaired health status in animals exposed to contaminants in estuarine ecosystems. This information can then be used to direct laboratory confirmation of the cause, if necessary (see, for example, Buckley et al., 1985; Gardner et al., 1991). Continued monitoring with these methods can be used to detect changes in a population's health during and following environmental intervention. Because changes at the organismal level precede changes in population and community characteristics, pathobiological studies can provide an early indication of the effectiveness of management actions.

Table 4-21. List of Pathobiological Terms

biomarker	any biological method used to detect the exposure of organisms to hazardous chemicals in the environment by measuring the response of the organisms to the contaminant through comparative molecular, biochemical, physiological, or anatomical observations of cellular dysfunction
cytochrome P450	a protein in the microsomes of liver cells that is important in catalyzing the metabolism of steroid hormones and fatty acids and in the detoxification of a variety of chemical substances
cytogenetics	the study of cytology in relation to genetics, especially the study of chromosomal behavior in mitosis and meiosis. Modern cytogenetics has led to the identification of chromosomes as bearers of the genes and deoxyribonucleic acid (DNA) as the key molecule of the gene
genotoxic agents	chemical and physical agents that can produce genetic alterations at subtoxic concentrations and can result in altered heredity characteristics. Genotoxic agents generally possess specified chemical or physical properties that facilitate their interaction with nucleic acids
hepatic	of, or relating to, the liver
histopathology	pathologic histology; the science or study dealing with the cytologic and histologic (microscopic) structure of abnormal or diseased cells, tissues, and organs in relation to their function
histochemistry	the study of the chemistry of cells and tissues using light and electron microscopy, special chemical tests, and special stains to determine the location of certain enzyme systems or reaction products in the cell
immunoassay	measuring the protein and protein-bound molecules that are concerned with the reaction of an antigen with its specific antibody, as in the detection of hormones or other substances
immunology	the study of being protected from a disease; the study of the response of the body and its tissues to a variety of antigens, including red cells, pollens, transplanted tissues, and even the individual's own cells
inclusion bodies	bodies present in the nucleus or cytoplasm of certain cells in cases of infection by filtrable viruses or as the result of degenerative diseases or exposure to chemicals
macrophage	cells of the reticuloendothelial system having the ability to phagocytose particulate substances and to store vital dyes and other colloidal substances
pathobiology	pathology, the study of disease, with emphasis more on the biological than the medical aspects of the essential nature, causes, and development of abnormal conditions, as well as the structural and functional changes that result from the disease process
smooth endoplasmic reticulum (SER)	a connecting network of tubules that course through the cytoplasm of the cell and can be viewed using the electron microscope; SER is essential to metabolic functions of the cells

SOURCES: *Stedman's Medical Dictionary,* 1982; *Taber's Cyclopedic Medical Dictionary,* 1985.

4.6.2 Monitoring Design Considerations

A field survey to collect target organisms and tissue samples may be required for pathobiological monitoring (Hargis et al., 1984; U.S. EPA, 1987b). In certain instances, a large sample size may be needed to establish statistical significance because of normal variation from animal to animal, species and generic differences, and migratory habits of fish. For these reasons, and the labor-intensive nature of pathobiological methods, the availability and allocation of funds, time, equipment, and trained personnel must be considered when planning to include pathobiological methods in monitoring programs.

Selection of Target (Indicator) Species

A key component of any pathobiological monitoring program is the selection of target species. The fundamental criterion is the ability to use the selected species to make comparisons between sampling locations and sampling periods. It is recommended that the target species possess the following characteristics:

- Abundant enough, temporally and spatially, to allow for adequate sampling;
- Large enough to provide adequate amounts of tissue for analysis;
- Sedentary (nonmigratory) in nature to ensure that pathobiological abnormalities are representative of the study area; and
- Easily collected.

It is also recommended that an existing database documenting exposures and sensitivities of the target organism specific to contaminants of concern is available.

Fish provide sufficient tissue for analyses and may indicate potential threats to human populations. However, fish are motile and pathobiological abnormalities detected may not be representative of the study area (see Vogelbein et al., 1990).

Bivalve molluscs, either attached to the substratum or burrowing in sediments, have been sampled extensively to examine the condition of these commercially important estuarine species (e.g., Mussel Watch, National Status and Trends program). The most common target species have been oysters (*Crassostrea virginica*) and mussels (*Mytilus* spp.), although pathobiological and bioassay studies have also been performed on other species, such as crabs and penaeid shrimp.

Secondary considerations, based on economic importance and status as a bioassay organism, may be applied to further winnow the list of candidate target species to a practical number of species to be analyzed.

Sampling Location

Appropriate locations of sampling stations depend on the objectives of the study. For example, to evaluate whether there is a statistically significant increase in lesions, stations should be located to collect specimens from contaminated and uncontaminated (background or control) areas for statistical comparison. It is also important to demonstrate a dose-response relationship between the pollutant concentration and incidence of tumors or other lesions by locating sample stations along a contamination gradient (i.e., from highly contaminated to moderately contaminated to uncontaminated). It is recommended that stations be located in areas where the geographic area of contamination is large enough that sampled fish could reasonably be expected to have spent a considerable amount of time within the influence of the pollutant (U.S. EPA, 1987b). Nielsen and Johnson (1984) and Cailliet et al. (1986) may be consulted for further fish sampling methodologies. Information on sampling bivalves and other invertebrates is contained in Couch (1978), Yevich and Barszcz (1983), Turgeon et al. (1991), and other sources.

4.6.3 Analytical Methods Considerations

Anatomic Pathology Methods

Anatomic pathology methods examine tissues with the naked eye (gross anatomic pathology), the aid of a light microscope (LM methods), or the electron microscope (EM methods). Gross anatomic methods are concerned with obvious adverse changes in tissue that can be observed in the field (Hunn, 1988; Hargis et al., 1984). The advantage of gross methods is that large numbers of specimens can be examined rapidly. However, the methods are generally nonspecific (i.e., it is not possible to determine that a specific pollutant led to a specific disease). An exception to this is cataracts in fish, which have been linked with polynuclear aromatic hydrocarbon pollution in the field (Hargis and Zwerner, 1988).

A disadvantage of anatomic pathology methods is that they require specialized personnel and laboratories. The methods are, however, generally standardized, routine, and operational in existing Federal, State, university, veterinary, and private diagnostic laboratories that specialize in aquatic animal pathology. Current activities are aimed at developing a field-to-laboratory response-diagnostic scenario in which adverse effects are found in the field, diagnosis is made in the laboratory, and experimental studies are initiated to verify results. Refer to Yevich and Barszcz (1980), Howard and Smith (1983), Johnson and Bergman (1984), Klontz (1985), Meyers and Hendricks (1985), and U.S. EPA (1987b) for more information on these methods and techniques.

Light Microscopy Methods

LM (histologic) methods use the light microscope to view cells and tissues. These methods can detect changes such as inflammatory responses, alterations in the appearance of cells, cancerous/precancerous lesions, and damage due to parasites (USEPA, 1986c). The advantages of LM methods are that they are organ-specific (i.e., the lesion can be localized to a specific organ) and can detect microscopic and subtle cellular alterations that are not evident on visual inspection. However, LM methods are more expensive (approximately $30/sample) and slower than gross methods.

Electron Microscopy Methods

EM methods use the electron microscope to detect changes in tissue at cellular and subcellular levels, such as the identification of the nature of inclusion bodies or changes in the amount of smooth endoplasmic reticulum. An advantage of EM methods is that they can be highly specific because the pollutant can be localized within certain parts of the cell. These inclusions may contain the causative chemical agent; lead, gold, iron, bismuth, uranium, beryllium, mercury, copper, and arsenic are a few of the metals that can be deposited intracellularly (Sorenson and Smith, 1981). Additionally, EM methods can be used to investigate subcellular mechanisms of pollutant action. The major disadvantage of EM methods is that they are very expensive (minimum $400/fish) and very slow, requiring highly skilled technical expertise.

Histochemical Methods

Histochemical methods use the microscope in conjunction with special chemical tests and stains to localize specific enzyme systems or reaction products in the cell. For instance, histochemical assays have been used on liver tissue during and after tumor formation to yield important information on the biochemistry of specific lesions (Hinton et al., 1988; Prophet et al., 1992; Sumner, 1988). Histochemical methods are reliable and can be highly specific for certain classes of organic compounds and metals. However, highly skilled technical expertise is required to carry out the methods in the laboratory.

In Vitro Tests

In vitro tests are generally more sensitive than whole animal systems, less expensive to carry out, and of shorter duration. However, in in vitro tests, defense mechanisms found in the intact animal are missing. In vitro systems offer the greatest flexibility for the testing and study of environmental contaminants. They can be designed to be relevant to the species of interest in a given area, and multiple types of measurements can be taken from a single test system (e.g., metabolic products, mitotic activity, cytotoxicity and genetic damage). However, comparative in vitro and in vivo studies are needed to correlate and relate changes that occur in each system (Landolt and Kocan, 1983).

Reproductive/Developmental Methods

Reproduction studies are designed to examine a number of parameters in the reproductive process: gross examination of the egg and numbers of eggs, embryo viability, the proportion of fertilized eggs (i.e., fertilization success), and larval development and viability (USEPA, 1986c). Studies that examine the egg itself incorporate microscopic methods that are time-consuming and expensive (West, 1990), but serve as a direct measure of reproductive success. Some methods deal with the egg at the molecular level and analyze the action of chemical and physical agents whose toxicity is directed toward genetic (DNA) components of the egg.

Different cytogenotoxic tests can be used to measure a diverse array of effects including gene mutation, chromosome damage (sister chromatid exchange), primary DNA damage, or oncogenesis (i.e., tumor formation and development) (Brusick, 1980; Landolt and Kocan, 1983; Klingerman, 1982; Shugart, 1990). Another area that is currently being investigated as a suitable, sublethal assay for toxicity of certain wastes in the marine environment is the fin regeneration test (Weis et al., 1990). The information collected using these methods can be used to assess the effects of pollutants on the reproductive capacity of animals. An understanding of these effects can be useful in evaluating observed population and community-level changes relative to the occurrence of specific pollutants.

Gonadotropic and steroidogenic hormones regulate the reproductive capacity of an organism. The level of these hormones has been used to assess how pollutants affect the reproductive capacity of fish (V. Varanasi, 1990, National Marine Fisheries Service, NOAA, personal communication). The analysis is a sensitive indicator of exposure affecting major biological processes that impact the whole population. However, relatively detailed information about the normal reproductive cycle of the animals is necessary to apply these methods in the field (V. Varanasi, 1990, National Marine Fisheries Service, NOAA, personal communication).

Biochemical Methods

Biochemical methods have been used in field studies to measure various indicators of environmental contamination. These methods are inherently sensitive and may provide basic information about early changes in response to environmental contamination at the cellular level. The development of a suite of indicators having both specific and nonspecific responses can provide information on the type of stressors, mechanisms of action, extent of physiological dysfunction, and potential long-term population consequences (Thomas, 1990).

Fish can respond to generalized stress, contaminants being one type of stress, through induction (increased synthesis) of stress proteins (Pickering, 1981; Sanders, 1990). Stress proteins are currently being investigated for use as generalized biochemical

indicators of stress in fish, chemical-class pollutant indicators, and mode of action indicators. The methods for detecting stress proteins involve radioisotopic and immunologic methods that measure the amount of stress protein present after a stress (i.e., exposure to a pollutant) occurs. At present, cDNA probes are being used experimentally to measure the correlation between stress and induction of stress proteins. These methods potentially afford a high degree of sensitivity.

It has been suggested that induction of the fish hepatic microsomal mono-oxygenase (MO) enzyme could serve as a sensitive biological indicator for certain classes of chemicals in water (Payne et al., 1987; Kleinow et al., 1987; Lech et al., 1982; Jimenez et al., 1990; Haux and Förlin, 1988). Metallothioneins (MT) have been under consideration for use as a monitoring tool for trace environmental metal pollution due to their induction as a result of exposure to certain metals (Engel and Roesijadi, 1987; Garvey, 1990; Haux and Förlin, 1988). However, additional scientific research is required to understand the basic biology of fish before the exact significance of field studies using these techniques can be ascertained.

A concern when measuring biochemical variables in fish to detect environmental pollutants is that their exact biological significance is rarely understood. In addition, for most of the biochemical variables studied, the normal range for a particular fish population and the factors influencing these variables are often unknown (Neff, 1985). Even with these limitations, biochemical methods hold considerable promise as sensitive early indices of exposure to environmental stressors (Thomas, 1990). However, additional research is needed so that simplified, more cost-effective field methodologies can be developed.

Immunological Methods

Immunological biomarkers are simple, sensitive, reproducible, and workable in the field (Weeks et al., 1990; D. Anderson, 1990; R.S. Anderson, 1990). These indicators provide supportive evidence for linkage between a stressor (toxicant, etc.) and disease outbreaks in fish and shellfish. The immune response can be used to monitor a specific antigen or microorganism responsible for pathological conditions in fish. Biologists can perform quick and sensitive assays in the field or in their own diagnostic laboratories because many immune assays are becoming available in kits (Rowley, 1990; Matthews et al., 1990). Many immunological assays do not require sacrifice of the animal. Blood samples can be taken periodically to follow the kinetics of the effects of stress in a single animal; however, the effects of handling stress on aquatic species must also be considered in this case. The immune response is physiologically similar among most vertebrates and similar equipment and materials can be used to test all species of fish as well as shellfish (see Anderson, 1987). There is a rapidly growing body of literature on immunotoxicology from veterinary and aquatic animal sciences.

The selection of immune system indicators for the study of stress effects depends on many factors, including specific study objectives, available equipment, training, personnel, and length and number of assays. The most sophisticated and sensitive assays are costly and require highly trained personnel, whereas simple assays can be performed by field biologists with only basic laboratory supplies.

A major limitation of immune indicators is that the response is sometimes too broad to provide conclusive evidence that the observed reaction is actually due to a specific complex to be considered. Cross-reactions and heightened responses to nonspecific factors may prevent the interpretation of assays with absolute certainty. The immune response in fish or shellfish will be distinctive for a specific antigen or disease-causing agent. This will frequently make it difficult to know which immune indicator is most affected and which immunological assay to apply. Expensive materials and laboratory equipment are needed for sophisticated immunological assays. Confirmatory assays are advisable to be sure that a particular stressor is the only cause of a particular effect. Many animals should be sampled because of natural variability among individuals.

Physiological Methods

Hematological methods have been used by biologists for many years to assess the general health of fish in hatcheries and research laboratories. The procedures are well-standardized (Blaxhall and Daisley, 1973; Wedemeyer and Yasutake, 1977) and are easy to carry out, even in the field. Hematological measures may be affected by the stress of capture, but are far less influenced than some other measurements, such as blood glucose (Larson et al., 1985). Hematologic methods have been successfully implemented in the field and have been rated as the best physiologic method for evaluation of pollutants (U.S. EPA, 1986c). For the measurement of hematological factors in fish, techniques similar to those used in human and veterinary clinical laboratories are generally used with minor modifications (Heath, 1987; Bouck 1984). Blaxhall and Daisley (1973), Wedemeyer and Yasutake (1977), and Ellis (1977) provide practical guides to the adaptation of these methods for use on fish blood.

The hematocrit/erythrocyte determination may not be as sensitive to pollution as is the leucocyte count, at least as far as its response to metals is concerned (Larson et al., 1985). It remains to be determined how sensitive the procedure is to subtle environmental changes. In general, chemical and physical stressors cause a decrease in the leucocrit, whereas infections produce the opposite response. However, it is also possible to obtain elevations in granulocytes concomitant with a decrease in lymphocytes, thereby yielding an unchanged leucocrit (Peters et al., 1980). Thus, the method has limitations for the detection of chronic stress (Wedemeyer et al., 1983).

Several obstacles, such as capture stress, limit the potential usefulness of physiological tests in field work. Capture stress precludes the use of sensitive, early indicators of environmental stress. The use of physiological responses of wild fish to assess

environmental quality is difficult because responses due to toxicants often cannot be distinguished from those induced during handling of the wild fish (Bouck, 1984; Folmar, 1993).

4.6.4 QA/QC Considerations

Pathobiological methods have a wide range of sensitivities and response times (i.e., when a response can be detected). For instance, certain immunologic, electron microscopic, and biochemical methods can detect early changes in cells and are very sensitive. Gross anatomic pathology methods, on the other hand, can detect cellular changes only after lesions can be seen and, therefore, are less sensitive.

General considerations for QA/QC procedures have been covered earlier in this document. With regard to pathobiological methods, it should be noted that careful and consistent handling of aquatic specimens is required to minimize trauma and confounding effects, such as exposure to air. Organisms should be held in the laboratory under conditions as near to those found at the site of collection as possible and for as short a time as practicable before performing assays. Fish and shellfish collected for histopathological examination must be properly fixed (e.g., immersed in a formaldehyde or glutaraldehyde solution) to stop metabolic activity. This may require opening or sectioning the organisms to allow the fixative to rapidly penetrate all tissues and preserve the cellular structure in its existing condition. The organisms must still be active or moribund, but not dead, before being fixed. Failure to follow proper fixation procedures will interfere with the interpretation of lesions in anatomic pathology studies.

As with other monitoring methods, samples must be accurately labeled at the time of collection and routine QA/QC procedures should be instituted, including tracking samples, carefully recording methods used, using fresh solutions, and treating both control and exposed samples equally. Whenever possible, organisms for each group to be tested should be of the same species, age, and sex. Sections of tissues and organs, or for small organisms, the whole animal, should also be prepared as uniformly as possible with respect to homogeneity and orientation so that microscopic observations can be made on the same organs and areas for each specimen. Subsamples of sections should be examined (blind) by another pathologist to confirm the diagnoses. See USEPA (1987b) for additional information on QA/QC procedures for histopathological examinations.

4.6.5 Statistical Design Considerations

Statistical strategies may mitigate the high costs of pathobiological monitoring methods. As discussed earlier, power analyses considering the strategy of compositing samples can often lead to a cost-effective monitoring design strategy. Power-cost analyses are necessary in selecting the appropriate sample/replicate number, sample location, and sampling frequency. If the primary objective of a monitoring program is to determine

pathobiological differences between sampling locations, composite sampling may be an appropriate strategy. However, a limitation of composite sampling is the inability to directly estimate the range and variance of the population of individual samples. Similarly, the use of space- or time-bulking strategies will severely limit a monitoring program's ability to assess spatial and temporal heterogeneity of the samples.

Given that the monitoring program must accommodate a fixed level of sampling cost, the best strategy for pathobiological monitoring is to collect more replicates at fewer locations. For histopathology, from 25 to 100 animals per species may need to be surveyed to detect low incidences of disease. The selection of appropriate statistical analyses must be made with these limitations in mind, as well as the specific types of statistical analyses that can be performed on different types of data. For example, quantitative information from measurements of enzyme levels or computerized image analysis/morphometric programs may be effectively analyzed by parametric statistics (if the data fit the conditions for normal distributions, limitations of assumptions, etc.), but histopathological observations may need to be rated on presence/absence or categorized qualitative scales that will require nonparametric techniques. USEPA (1987b) provides additional information on the types of sampling designs and statistical tests that may be appropriate for these data.

4.6.6 Use of Data

Data Interpretation

Data interpretation for pathobiological methods may be limited because there are relatively few trained personnel, facilities and equipment may be expensive, and references for newly emerging techniques may be scarce. However, because of the recent interest in developing biomarkers for monitoring the effects of natural and anthropogenic environmental stresses on aquatic organisms, many research programs are under way at Federal, State, and academic facilities to develop, standardize, and validate the most promising biomarkers for sentinel species that will establish cause-and-effect links for pollutant exposure. Basic laboratory research and experimental studies must be conducted in conjunction with field work to elucidate the relationships between contaminant levels, structural, biochemical, or functional pathologies, and population health (Johnson and Bergman, 1984; McCarthy, 1990). A critical concern is the coordination of efforts and creation of a multidisciplinary approach.

It is important to establish baseline data on selected species, as well as to demonstrate the alterations in the health of those species due to contaminant exposures. Methods and techniques, terminology, and interpretation of lesions and effects must be standardized. In addition to a rapidly expanding body of literature on baseline measures of health, histological atlases for several species of fish and shellfish, and courses, workshops, meetings, and special symposia (e.g., Responses of Marine Organisms to Pollutants/Woods Hole, MA, and Plymouth, England; Annual Aquatic Toxicology

Workshop, Canada), several professional societies (e.g., Society for Invertebrate Pathology, American Fisheries Society/Fish Health Section) are facilitating training in techniques and communication among investigators and laboratories. Diseases of fish and shellfish have been reviewed by several authors (e.g., Sparks, 1985; Ferguson, 1989; Roberts, 1989; Sindermann, 1990; Couch and Fournie, 1993). For histopathological studies of tumors in fish, standardization of nomenclature will be available in Dawe et al. (in press).

As for any monitoring program, long-term studies of health and disease in aquatic organisms will aid in identifying and interpreting observed pathobiological effects. Furthermore, it will be important to archive data so that they will be available for future comparisons. The Registry of Tumors in Lower Animals at the National Museum of Natural History, Smithsonian Institution, Washington, DC, houses over 3,500 cases of neoplastic and nonneoplastic lesions representing a wide variety of host aquatic species, pathogens, and environmental stresses from field and laboratory studies conducted around the world. The Registry also contains the Registry of Marine Pathology, originally developed by the National Marine Fisheries Service (NMFS), and the collection of crustacean histopathological material researched by Dr. Phyllis T. Johnson, NMFS. Although primarily serving as a clearinghouse for information on neoplastic diseases and research, the Registry is also able to direct inquiries for information on nonneoplastic diseases, and the materials archived there are available for study by qualified investigators. Data collected from the long-term monitoring efforts of NOAA's National Status and Trends Program (NS&T) and EPA's Environmental Monitoring and Assessment Program (EMAP) will also provide useful information for the interpretation of the various pathobiological methods that are being employed in these studies.

Data Integration

Measuring or evaluating the effects of stressors on fish and shellfish by pathobiological indicators is difficult primarily because of the large number of variables that can influence biological response. Variables including water temperature, nutritional status, species, sex, reproductive and developmental stages, and physiological functions can render tests difficult to compare and evaluate.

Anderson (1990) outlined a plan including four levels of study in a tiered approach to investigate the effects of stress on the immune system and to quantify the possible contribution of these environmental and physiological variables on the stress response. The plan, generally speaking, appears to be applicable to all pathobiological methods and may be adapted to them using the following four levels: (1) observations of fish and shellfish populations in the field; (2) studies of caged fish or shellfish in the field; (3) in vivo exposures in the laboratory; and (4) in vitro assays in the laboratory.

The first level of study includes the collection of information from field observations on relative abundance, reduction in sport-fish catches, or decline in commercial harvests. Reduced yields reported by anglers in previously productive fishing grounds may be correlated with environmental stressors or pollutants occurring at these sites. If sick or moribund fish or shellfish are called to the attention of local biologists by anglers, gross morphological descriptions might be made and isolation of the disease agent attempted.

The second level of investigation involves the use of caged fish or shellfish placed in the field to test for the presence or action of environmental stressors. Groups of fish or shellfish can be placed in pens or cages at suspected sites and their responses (signs of disease, acute mortality) can be compared with those of control fish or shellfish caged in unstressful areas. Organisms from the cages can be sampled at various intervals and the intensity of various immune responses quantified as a function of the time the fish or shellfish were exposed. Levels of immune responses can be compared by injecting specific disease agents into both control and test organisms, and recording disease and death rates.

The third level of assay is in vivo laboratory tests, by which immune response can be evaluated in experiments with calibrated dilutions of specific contaminants and kinetic measurements of the immune response at each dilution. Challenges with disease agents can be more easily controlled in the laboratory to provide information on how the stressor makes the fish or shellfish more susceptible to specific pathogens.

A fourth level of investigation is the recently developed method for testing the effects of chemicals, drugs, and other stressors in vitro. Spleens and other immunopoietic organs can be removed from fish and placed in tissue culture media and their reactions to pollutants tested using the passive hemolytic plaque assay (Anderson et al., 1986). Use of this method to measure the effects of stressors allows maximal control of the levels of pollutants. Because the immune response is monitored under defined laboratory and environmental conditions, important information is obtained about how specific stressors affect the immune response. In vitro methods require fewer fish than in vivo methods because tissue and organ samples can be divided into many sections, which also reduces the variability of responses.

McCarthy (1990) presented a research strategy to validate biomarkers and provide the scientific understanding necessary to interpret biomarker responses. An evolving monitoring program was proposed that focused broadly on evaluation of contamination in an array of ecosystem types. The challenges and obstacles to be addressed in such a program include the following:

- The quantitative and qualitative relationships between chemical exposure, biomarker response, and adverse effects must be established.

- Responses due to chemical exposure must be distinguishable from natural sources of variability (e.g., ecological and physiological variables, species-specific differences, and individual variability) if biomarkers are to be useful in evaluating contamination.
- The validity of extrapolating between biomarker responses measured in individual organisms and some higher-level effect at a population or community level must be established.
- The use of exposure biomarkers in animal surrogates to evaluate the potential for human exposure should be explored.

The plan is an ambitious multiyear research and development program involving the formulation of a long-term, interagency, interdisciplinary activity such as the Environmental Monitoring and Assessment Program (EMAP) by EPA in cooperation with other Federal agencies.

4.6.7 Summary and Recommendations

Rationale

- Pathobiological methods provide information concerning biological organ systems that, through an evaluation of organ structure, activity, and function, can be used to determine adverse effects of pollutants in the environment.
- Pathobiological methods should be used in concert so that cause-and-effect relationships can be evaluated.

Monitoring Design Considerations

- Sampling stations should be located along a contamination gradient (i.e., from highly contaminated to uncontaminated). This type of sampling strategy will allow dose-response relationships to be evaluated.
- Fish pathobiological monitoring should be conducted only if the target species could reasonably be expected to have spent a considerable amount of time within the area of contamination.
- Large sample sizes will frequently be required for fish pathobiological monitoring due to natural variability among individuals and taxa.

Existing Analytical Methods

Anatomic Pathology Methods

- Tissues are examined with the naked eye or the aid of a microscope

- Light microscopic (LM) methods
  - detect changes at the cellular level
  - organ and time-series specific
- Electron microscopic (EM) methods
  - detect subcellular changes
  - specific as to area of cell or site of pollutant action
- Histochemical methods
  - detect enzyme systems or reaction products in the cell
  - specific to chemical class

Reproduction/Development Methods

- Reproductive capacity is reflected in population recruitment and abundance
- Methods that examine the egg itself provide a direct measure of reproductive effects from pollutants
- Cytogenetic (DNA) tests
  - in vitro tests offer greatest flexibility in terms of sensitivity, expense, and time required and are frequently conducted for convenience and availability.
  - sister-chromatid exchange (SCE) assays are DNA damage tests used as a screening tool and are dose-responsive and sensitive to low concentrations of pollutants.
  - the aneuploidy technique is simple and easy to use but is inaccurate for sublethal effects.

Biochemical Methods

- Several methods are specific for a certain compound class.
- Use a suite of indicators for environmental monitoring.
- Microsomal mono-oxygenase (MO) assays could serve as sensitive biological indicators for certain chemical classes.
- Metallothionein (MT) assays are nonspecific for metal exposure, but can provide information on the likelihood of a particular metal pool producing a pathological effect.
- Stress proteins are not pollutant-specific, but could result from generalized stress.

Immunologic Methods

- Immunologic assays can provide information on pollutant-induced stress effects; however, they may require confirmatory assays.
- Many animals should be sampled because of natural variability among animals.

Physiologic Methods

- Serious "interferences" can be caused by stress induced during collection and may limit the potential usefulness of physiological tests because effects of toxicants cannot be distinguished from those induced during handling of the wild organisms.
- Hematologic methods for fish, such as measurements of hematocrit (packed cell volume), hemoglobin concentration, erythrocyte count, and leucocyte count (or volume), can be successfully used. Although the fish are not immune to stress of capture, hematologic methods are influenced far less than some other measurements such as blood glucose.
- The leucocrit can be a more sensitive measure of metal pollution than the hematocrit.

QA/QC Considerations

- Pathobiological methods have a wide range of sensitivities.
- Organisms must be carefully handled and properly prepared for each method.
- The main areas of concern with regard to analytical QA/QC are precision, accuracy, representativeness, completeness, and comparability.

Statistical Design Considerations

- Composite tissue sampling consists of mixing tissue samples from two or more individuals collected at a particular location and time.
- Space-bulking (combining composites from several locations) and/or time-bulking (combining several composites over time from one location) strategies should be used judiciously since information concerning spatial and temporal heterogeneity may be lost.
- Power analyses have shown that for a fixed level of sampling effort, a monitoring program's power is generally increased by collecting more replicates at fewer locations.

- The appropriate statistical tests must be performed and may vary depending on the type of data generated.

Use of Data

- Data use and interpretation may be limited by relatively few trained personnel and expensive equipment, but many research and training programs are now under way at Federal, State, and academic facilities to improve biomarker methods in sentinel species.
- Basic laboratory research must be conducted and biological methods must be tested in the field.
- Information communication
  - need to coordinate efforts, communicate, and create a multidisciplinary approach to relate lab and field studies and establish cause-and-effect relationships.
- Data integration
  - long-range research strategies should be followed to validate biomarkers and provide scientific understanding necessary to interpret biomarker responses.

4.7 FISH POPULATIONS

The objective of monitoring fish populations is to detect and describe spatial and temporal changes in the abundance, structure, and function of fish communities. To attempt to protect and preserve healthy fish from the possible effects of pollutant discharges, estimates of fish population abundance and detailed knowledge of fish life histories are required.

Except in the most severe cases, it is usually impossible to directly link pollutant loadings to stock levels (i.e., there is a poorly established cause-and-effect relationship between pollution and fish population responses). Natural population fluctuations in fishery stocks will usually be much greater than effects due to pollution. The effects of discharges are obvious when fish are killed outright by high concentrations of toxic substances (Rothschild, 1986). However, these instances are rare compared to the prevalent situation, in which fish are exposed to lower pollutant levels that may not be lethal but are thought to be harmful in some sense. Sublethal pollutant loadings can be expressed in terms of increased body burden of toxicants or greater incidence of various lesions. The effects of sublethal concentrations on growth, mortality, and reproduction are not known (Rothschild, 1986) As a result of these limitations, fish population measurement methodologies require further refinement and field validation before they can be promoted for regular use in 403 monitoring or permit decisions.

4.7.1 Rationale

Under section 403 of the Clean Water Act, permitters must use 10 guidelines in determining whether a discharge results in unreasonable degradation or irreparable harm to the marine environment. Fish population studies have generally not been used to directly link a source of pollution to observed responses. However, some recent studies have indicated that selection of resident bottom fish as indicators of pollution can support other data linking sources of pollution to environmental effects, especially if such studies are conducted in conjunction with benthic studies and parallels can be drawn between the two. In addition, such methodologies do address the following three 403 guidelines:

- Composition and vulnerability of potentially exposed biological communities;
- Importance of the receiving water area to the surrounding biological community; and
- Existing or potential recreational and commercial fishing.

Population effects can include those caused by changed reproductive rates or changed distribution and migration patterns. Effects of suppressed reproductive rates on population density have been clearly demonstrated in the diminishing populations of the striped bass in San Francisco Bay. The suppressed reproductive rates have been shown to correlate with toxic concentrations in the bay (Whipple et al., 1984). Several

laboratory studies have demonstrated that certain fish can avoid toxics, including DDT, endrin, and Duroban. There is, however, no proof that fish avoid toxicants in the field. If they do, "hotspots" would be avoided by such species and the population would suffer less exposure to toxic effects.

One of the greatest problems in analyzing effects of toxics on populations is separating direct toxic effects from natural or non-pollution-related variations including climatic changes and overfishing. Results of population studies must be evaluated together with measurements of other environmental parameters before any cause-and-effect relationship can be established.

4.7.2 Monitoring Design Considerations

Specimen collection, analysis, and evaluation of fish community structure and function are typically time-consuming, labor-intensive, and expensive tasks. A survey vessel manned by an experienced crew and specially equipped with gear to collect organisms is required. Expert taxonomists are needed to identify and enumerate collected fish specimens.

The results of fish monitoring programs can vary substantially depending on the objectives and corresponding design specifications. The characteristics primarily responsible for the variability in the results are the following:

- Type of sampling gear,
- Volume sampled, and
- Location and timing of sample collection.

It is essential to understand the effects of these monitoring design characteristics on the results and to standardize them as much as possible to ensure the comparability of sampled data. Analyses of power-cost efficiencies are useful in selecting the appropriate sampling gear and sample processing protocols. Ferraro et al. (1989) provide an example of power-cost analyses.

Sampling Devices

Sample collection protocols influence all subsequent laboratory and data analysis; it is key that fish population samples be collected using acceptable and standardized techniques. Several types of devices can be used to collect fish samples: traps and cages, passive nets, trawls (active nets), and photographic surveys (Fredette et al., 1989). Many of these devices selectively sample specific types of fish. Accordingly, conducting comparisons between data collected using different devices and even different nets of the same type is inadvisable.

Traps and Cages

Traps and cages are usually designed to attract and capture specific organisms. They are useful in studies examining the activities of a particular target organism in a given area. Traps and cages provide only qualitative measures of organisms in a particular area.

Passive Nets and Trawls

Nets vary in their selectivity of the species that are captured and in the efficiency of retaining captured specimens. The size of the net, its configuration and orientation, and the avoidance behavior of the target species should be considered when using any net. The mesh size affects the speed at which the net can be towed, as well as the size of fish caught. The slower the towing speed, the more likely that some organisms will either avoid or escape the net. It is highly recommended that the mesh size, net opening, and duration, direction, and speed of towing be set in order to compare trawls. In any monitoring program, otter trawl net size must be kept constant to ensure intercomparison of sampling results.

Passive nets (e.g., gill nets) are deployed at a fixed position; organisms become entangled or trapped within the netted area. Passive nets are used to collect selected target species and to provide a qualitative means of sampling fish populations. Limitations associated with passive nets include: .

- Nets, ordinarily, must remain in place for an extended period of time.
- Deployment and recovery of nets are typically time-consuming processes.

Trawls (active nets) are drawn through the water, and results are more immediate than those obtained through the use of passive nets. Trawls are typically used to collect large quantities of fish at various depths (Fredette et al., 1989).

Photographic Surveys

Photographic surveys are effective when the bottom topography is uneven or trawling is not possible. The utility of photographic surveys is limited by water clarity, difficulties in identifying species, and fish avoidance of the camera system. In addition, further studies comparing photographic surveys to trawls are required before comparisons may be made. This method is also limited by the qualitative nature of the data (Fredette et al.,1989).

Volume Sampled

Different species of fish have different scales of horizontal and vertical spatial distribution (Cushing, 1975; Bond, 1979). Costs of laboratory analysis of the sample increase with increased volume sampled. Analyses of spatial and temporal scale,

statistical power, and costs will assist in determining optimal sample volume. It is highly recommended that a standard sample volume (same tow duration, tow speed, and net opening area) be analyzed to ensure data comparability (Green, 1979).

Selection of Sampling Period

Fish assemblages are dynamic; the most common temporal patterns observed in fish communities are those associated with daily activity patterns (diurnal), seasonal changes, and life history strategies. To minimize energy expenditure, most fishes mimic the diurnal activities of their prey. These diurnal fluctuations typically occur in the vertical scale and should be considered to ensure collection of representative samples. Seasonal variation in fish assemblages may be due to changes in physical, chemical, and/or biological parameters: i.e., temperature, light transmissivity, dissolved oxygen, predation, reproductive stage, recruitment, etc.

Given the seasonal variation characteristic of fish assemblages in general, it is recommended that direct comparisons between samples collected during different seasons be avoided. Studies investigating interannual variation in the characteristics of fish communities should be conducted during the same season (preferably the same month) each year.

4.7.3 Analytical Methods Considerations

There are a variety of approaches to assess the effects of anthropogenic perturbance on fish communities of the marine environment. These assessment approaches may be grouped into three categories :

- Biological indices,
- Indicator species, and
- Multivariate analyses.

There has been little consensus among biologists regarding the suitability of various techniques for describing community characteristics and/or for assessing impacts. A critical evaluation of the use of biological indices to detect environmental change is presented in an EPA Technical Support Document (USEPA, 1985d). The indices, shown in Table 4-22, are evaluated on the basis of the following criteria :

- Biological meaning,
- Ease of interpretation, and
- Sensitivity to community changes due to anthropogenic sources.

The results of these evaluations and additional information on other analytical methods are summarized below.

Table 4-22. Biological Indices

Index/Method	Biological Characteristic Measured
Bray-Curtis (Curtis and Peterson,1978)	Dissimilarity
Dominance (Swartz et al.,1985)	Community structure
Number of individuals (USEPA,1985d)	Total abundance
Number of species (USEPA,1985d)	Total taxa
Biomass (USEPA,1985d)	Standing crop
Margalef's SR (Margalef,1969)	Diversity
J (Pielou,1966)	Evenness
Shannon-Wiener H' (USEPA,1985d)	Diversity

Biological Indices

The number of individuals and the number of species have been used as indicators of anthropogenic disturbance, as well as other environmental stresses (USEPA, 1985d). Furthermore, these simple biological indices are less ambiguous and can often be as informative as diversity indices (USEPA, 1985d; Green, 1979; Hurlbert, 1971). Measures of biomass have inherent problems in the collection of the data, e.g., loss or gain of weight due to preservative medium, drying times, or evaporative weight loss.

More complicated indices–e.g., species diversity, species richness, dominance, evenness–have found varying degrees of acceptance. Because of this, these methods should be used in conjunction with other assessment methods to help verify results. Diversity indices, which are measures of the distribution of individuals among species, have the following limitations (Green, 1984b):

- They often lack biological meaning;

- They are not robust empirical indicators of any important correlates of environmental health;
- They do not incorporate information on form and function of resident species; and
- They are susceptible to biases associated with well-described taxa.

Even so, species diversity indices are a widely used measure of community structure. Although diversity may not be able to pinpoint a single problematic discharge in an area that contains many, it can be an indicator of degraded water quality. Species diversity in fish communities is noted to increase with increasing distance from a pollution source (Armstrong et al., 1970; Bechtel and Copeland, 1970; Tsai, 1968). In fact, Bechtel and Copeland (1970) found a linear relationship between the volume of toxic effluent entering the Houston Ship Channel and the fish diversity of shallow stations.

The dominance index is a measure of the degree to which one or a few species dominate the community. The dominance index, herein defined as the minimum number of species required to account for 75 percent of the total number of individuals, has been useful in describing community structure (Swartz et al., 1985). Its advantages are that it is easily calculated, it does not assume an underlying distribution of individuals among species, and it is statistically testable.

Indicator Species

The evaluation of abundances of individual indicator species is generally informative and may reduce the cost of the analysis. The absence of pollution-sensitive species and the enhancement of opportunistic and pollution-tolerant species may assist in defining the spatial and temporal extent and magnitude of impacts. A preponderance of unspecialized feeders indicates areas of stress. However, indicator variables must possess the following characteristics (Green, 1984b):

- They must provide a sufficiently precise and accurate appraisal of :
  - species of concern,
  - anthropogenic disturbances to communities, and
  - presence/absence or magnitude of anthropogenic perturbance to the ecosystem.
- They must provide a cost-effective and statistically reliable alternative to monitoring all critical measures of habitat perturbance.
- They must be appropriate for the spatial and temporal scale demanded by the study objectives.

Further studies of the response patterns of fish species subjected to anthropogenic perturbations caused by discharges are required in order to select appropriate indicators of environmental impact. If a suitable indicator species is identified, it is desirable to

monitor the status and trend of that species' population. This will also be true of certain species having high economic or public value (e.g., striped bass). A wide variety of methods can be used to measure the size of fish populations and assess population structure. These include mark and recapture techniques and various techniques used to determine population sex and age structure. Nielson and Johnson (1984) and Cailliet et al. (1986) provide thorough discussions of these techniques and their applications. Ricker (1975) provides an excellent discussion of methods for the sampling of fish populations.

Statistical Analyses

Numerical classification encompasses a wide variety of techniques that have been used in the analysis of fish data to distinguish groups of entities (e.g., sample locations) according to similarity of attributes (e.g., species). These techniques differ from most multivariate methods in that no assumptions are made concerning the underlying distributions of the variables. Detailed descriptions of numerical classification analysis can be found in Romesburg (1984), Clifford and Stephenson (1975), Boesch (1977), Sneath and Sokal (1973), and Anderberg (1973). Boesch (1977) is particularly valuable as an introduction and guide to the use of numerical classification analysis in marine environmental studies. Guidance on the interpretation of classification results is provided in an EPA Technical Support Document (USEPA, 1988d).

Ordination analyses have also been used to reduce the dimensionality of the data set while maintaining the relationship between similar and dissimilar entities. At present no single ordination technique has been shown to be clearly superior for the analysis of biological data (USEPA, 1985d).

Multivariate analyses are effective heuristic tools. They generate visual representations that often indicate where further analyses ought to be conducted.

Analytical Approach Recommendations

Some of the most informative measures of community structure are the simplest (Table 4-22):

- Number of individuals,
- Number of species,
- Dominance,
- Abundance of pollution-sensitive species, and
- Abundance of opportunistic and pollution-tolerant species.

These indices have proved to be useful over various habitats and regions in assessing changes in fish population dynamics (USEPA, 1985d). Values of these variables may be determined from the list of species abundances generated during the taxonomic identification of collected specimens. Furthermore, the values of these variables may be easily tested statistically using parametric or nonparametric techniques. It is recommended that no single index or analytical method be used to assess impacts; rather, the assessment of impacts should incorporate information that each variable and method contributes concerning community structure.

Selection of reference sites is key to the evaluation of environmental impact assessment of point source dischargers. Results of analyses using reference measures provide the means of comparison by which anthropogenic impacts are detected. It is essential that selected reference sites exhibit similar habitat characteristics compared to monitoring program sampling sites. Several reference sites may be required to meet these criteria.

4.7.4 QA/QC Considerations

Sample Collection

Nets should be inspected for wear and tear leading to sample loss.

Taxonomic Identification

A key QA/QC issue is taxonomic standardization. Consistent taxonomic identifications are achieved through interaction among taxonomists working on each major group. Participation of the laboratory staff in regional taxonomic standardization programs is recommended to ensure regional consistency and accuracy of identification.

A sampling of the verified specimens should then be placed in a permanent reference collection. All specimens in the reference collection should be stored in labeled containers that are segregated by species and sample. Reference specimens should be archived alphabetically within major taxonomic groups.

4.7.5 Statistical Design Considerations

Consideration of statistical strategies will mitigate the high costs of collecting and processing samples. Power-cost analyses are necessary in selecting appropriate sample/replicate number, sample location, and sampling frequency.

Temporal Stratification of the Data

The time of the year should be controlled or stratified in the design; the use of annual averages is seldom good practice. Temporal stratification of the data should not be attempted until sufficient knowledge of long-term natural cycles is attained. Initially, simple regression analyses may be conducted on seasonally stratified data to identify

monotonic temporal trends. Whether conditions are improving or degrading over time may be further examined using various statistical time series analyses (e.g., temporal autocorrelation, spectral analyses).

Statistical Power

Selection of the number of replicates is an important component of program design. The inherent patchy distribution of fish communities requires collection of sufficient replicate samples to ensure an accurate description of the area. However, increases in replication increase sample processing costs. Power analyses assist in the allocation of sampling resources (stations, replication, and frequency) with regard to program finances and design.

Power analyses may be applied to determine the appropriate number of sample replicates required to detect a specified difference (USEPA, 1987d). The number of replications required to detect a specified minimum difference is a function of the statistical power and the variance in the data. Power analyses require a priori knowledge of the variability in the data. A best guess or, preferably, variation observed in historical data is often used initially in the design of the monitoring program.

To improve the power of a statistical test, while keeping the significance level constant, the sample size should be increased. Because of constraints in cost and time imposed by the monitoring program, however, this option may not be available. Power analyses have shown that for a fixed level of sampling effort, a monitoring program's power is generally increased by collecting more replicates at fewer locations. The number and distribution of sampling locations required to evaluate the effectiveness of the monitoring program will depend on the size and complexity of the discharge and the surrounding environment.

4.7.6 Use of Data

Monitoring of fish community structure provides a measure of the health of the marine habitat and can be used as a tool in the evaluation of spatial and temporal effects of marine discharges. The presence or absence of certain fish is useful in indicating the condition of the environment. Monitoring of fish communities also provides data required in the design and validation of fish population dynamics models and the selection of biological indicators (USEPA, 1990d).

In addition, monitoring of fish communities may directly provide accurate information essential in assessing the effectiveness of pollution abatement programs. Analyses of fish communities may be effectively used to monitor long-term change in the receiving environment (Cushing, 1975).

Fish serve as effective indicators of overall environmental condition, delineating the magnitude, spatial extent, and temporal trends of anthropogenic and natural perturbations to the ecosystem. Monitoring of fish will provide relevant accurate data fundamental to achieving the objectives of many marine monitoring programs. Such measurements, however, will not provide information linking specific pollution sources to observed effects. The results of the fish stock monitoring program can also be unclear as a result of the mobility of fish and their range of natural fluctuation. The data can be used to augment other monitoring data to obtain a clearer picture of the discharge effects.

4.7.7 Summary and Recommendations

Rationale

- The objective is to detect and describe spatial and temporal changes in the size, structure, and function of fish communities to protect the economic, recreational, and aesthetic value of the resident fisheries.
- Monitoring provides in situ measures of habitat quality and is a powerful tool in assessing environmental impact.

Monitoring Design Considerations

- It is recommended that the types of sampling gear, the volume sampled, and the location and timing of sample collection be consistent to allow for comparisons among studies.
- To reduce the variation due to seasonal differences, sampling should be conducted during the same season—preferably the same month—each year.

Analytical Methods Considerations

- It is recommended that simple measures of community structure be used to assess the condition of the marine fish: number of individuals, number of species, dominance, abundance of pollution-sensitive species, and abundance of pollution-tolerant species.
- Indicator species should possess the following characteristics:
  - sensitive to perturbances of concern;
  - cost-effective and statistically reliable alternative to measuring all species in a monitoring program;
  - statistically reliable indicative measures of habitat perturbance; and
  - appropriate for the spatial and temporal scale demanded by the study objectives.

- Selection of reference sites is key to the evaluation of environmental impact due to discharges; several reference sites may be required to provide proper control for sampling sites.

QA/QC Considerations

- Taxonomic standardization is key to the analysis of community structure. Recommended protocols include consistent interactions among taxonomists, reidentification of selected samples, and use of a reference collection.

Statistical Design Considerations

- Power analyses may be applied to determine the appropriate number of sample replicates required to detect a specified difference, thereby optimizing the high costs of collecting and processing samples.

Use of Data

- Data provide essential information to assess impacts due to anthropogenic perturbance, monitor recovery of the receiving environment, and validate community and population models.

4.8 PLANKTON: BIOMASS, PRODUCTIVITY, AND COMMUNITY STRUCTURE/FUNCTION

Although increased primary production resulting from intentional nutrient inputs has been shown to increase fish stocks in some experimental systems, the possible increase in fisheries in naturally productive systems is considered insignificant. In fact, it is more likely that stocks will decrease due to modification of the plankton species composition. Biogeographical distributions of plankton are determined by specific environmental factors such as light, temperature, salinity, and nutrients. Plankton vary in size over a range of seven orders of magnitude. Each predator species can utilize only plankton within two or three orders of magnitude in size, at the most, as a food resource. Additionally, the nutrient values of various prey differ so that a change in species composition to less nutritive species may cause stress on the predator species.

The purposes of monitoring plankton characteristics are (1) to identify the dominant species; (2) to detect short- and long-term spatial and temporal trends in overall biomass and productivity and species abundance, distribution, and composition; and (3) to examine the relationship between water quality conditions and these characteristics.

As was the case for fish population monitoring methods, measurements of plankton biomass, productivity, and community structure/function are not sufficiently developed to be used in a discharge-specific monitoring framework for section 403 assessments at this time. These measurements suffer from a lack of a specific cause-effect relationship between pollution and plankton responses.

4.8.1 Rationale

Although not directly applicable for use in monitoring the site-specific impacts of individual point source discharges, plankton measurements can be used to evaluate 3 of the 10 guidelines presented in the section 403 regulations for assessing the potential for unreasonable degradation:

- Composition and vulnerability of potentially exposed biological communities;
- Existing or potential recreational and commercial fishing; and
- Importance of the receiving water area to the surrounding biological community.

Changes in nutrient concentrations in coastal waters may result in the potential for long-term biological changes in the plankton community that may lead to changes in species distribution and abundance (both primary producers and consumers). A concern of the section 403 program is that excessive nutrient inputs can result in excessive phytoplankton biomass, which in turn can lead to increased turbidity and/or

changes in phytoplankton species composition and perhaps the trophic structure. If, based on water quality monitoring data, changes in trophic structure are suspected of occurring, periodic monitoring of the plankton community may be required.

The plankton component of a monitoring program could be used to provide data necessary to assess the effectiveness of management programs in mitigating potential adverse effects due to changes in the plankton community biomass and structure/function.

4.8.2 Monitoring Design Considerations

Plankton

Plankton monitoring strategies should be able to delineate between natural or seasonal variability in plankton stocks and variations caused by changes in nutrient concentrations. Characterization of phytoplankton taxonomic abundance and distribution and primary productivity provides indications of water quality conditions. Monitoring changes in phytoplankton population composition and densities is critical for the interpretation and evaluation of long-term trends in water and habitat quality. Further understanding of the causes of excessive water column and sediment oxygen demand requires tracking of photosynthetic activity and metabolic rates over time (Chesapeake Executive Council, 1988b). Zooplankton abundance and distribution are affected by both changes in phytoplankton and changes in predator populations. Therefore, population characteristics of this group can indicate symptoms of water quality problems, fishing pressure, and other habitat problems for predator species (Chesapeake Executive Council, 1988b).

Selection of Temporal Sampling Strategies

Because of their short turnover times, phytoplankton communities may respond to perturbations much more rapidly than other biotic groups. Therefore, phytoplankton samples should be collected relatively more frequently. In those situations where phytoplankton communities display pronounced seasonal variations in standing stock or production, it may be appropriate to use a temporally stratified sampling approach. For example, in the Maryland Chesapeake Bay Phytoplankton Monitoring Program, sampling takes place once monthly from October through March and twice a month from April through September (USEPA, 1989c).

The life span of zooplankton, on the other hand, is longer than that of phytoplankton, so the capacity for responding to perturbations is less than that of phytoplankton. Therefore, less frequent sampling is required. As with the phytoplankton community, the zooplankton monitoring program should consider the natural temporal fluctuations in abundance and species composition.

In many cases, regular monitoring of the zooplankton community may not be necessary unless changes in the phytoplankton community that would induce changes in the herbivore community are observed. Because many zooplankton graze on phytoplankton, in areas where the phytoplankton community has been affected, alterations of the zooplankton community are a distinct possibility. In other cases, monitoring of zooplankton may be desirable only when there is evidence of previous impact on the zooplankton community or in those situations where point and nonpoint source discharges are located in areas where there is high potential impact on zooplankton (for example, in environments with macroplanktonic larvae of important commercial or recreational species).

Sampling Methods

Phytoplankton

Phytoplankton samples should be collected at a variety of depths throughout the water column, including some above and some below the pycnocline. For example, composite water column samples collected for the Virginia Chesapeake Bay Plankton Monitoring Program are taken from five depths above and five depths below the pycnocline (USEPA, 1989c). Vertical collection of chlorophyll *a* (an indirect measure of phytoplankton standing stocks) can be examined at such stations through the collection of water samples with water bottles at various depths followed by fluorometric or spectrophotometric determination of chlorophyll *a*. If available, a pump station may be used with a flow-through fluorometer for a continuous profile of chlorophyll *a* concentration with depth (Lorenzen, 1966). Samples to be used for taxonomy analysis should be collected with water bottles because agitation associated with pumping may damage cells, making them unidentifiable. Pumps should be used only for determination of chlorophyll *a* concentrations.

Continuous vertical profiles of chlorophyll *a* concentrations can be obtained by attaching a fluorometer to a conductivity-temperature-depth (CTD) system. An additional sensor measuring dissolved oxygen can be attached to the CTD system. This combined instrument package will make direct measurements of chlorophyll *a* levels and water column stratification. The advantage of this measurement system is that instead of pulling water up from depth to a measuring device on the deck of a ship, the instrument is lowered through the water column, taking samples in undisturbed water. Sampling rates can be as high as 24 samples per seconds with a locating speed of 1 meter per second. Data are usually collected by a computer-based data acquisition system; in advanced systems, data can be displayed in real time as they are being collected. A rosette water sampler can be lowered at the same time as the CTD package to collect water samples at discrete depths. These samples will be used for taxonomy analysis and to verify and calibrate chlorophyll *a* measurements from the CTD fluorometer. The calibration will be carried out using laboratory spectrophotometer methods.

Zooplankton

Because zooplankton possess varying degrees of swimming ability, they have the potential for aggregating in patches or in a narrow depth strata. This introduces additional complications into quantitative sampling. It also means that zooplankton are able to avoid certain types of gear. Until recently, the role of the microbial loop, which consists of bacteria, flagellates, ciliates, and microzooplankton (<200 µm), has been overlooked. These organisms may represent a significant pathway in the reutilization and conversation of dissolved organic carbon into larger zooplankton and benthic organisms (Azam et al., 1983; Pomeroy, 1984). The sampling methods to be used for collecting zooplankton will vary depending on the size of the organisms. Microzooplankton (size range of 20 - 200 µm) can be collected with water bottles at various depths similar to those used for phytoplankton (Jacobs and Grant, 1978), or small (for example 44 µm) mesh nets can be used. Pumping systems can also be used (Beers et al., 1967). These have the advantage of being able to take samples while the ship is under way, but they may damage soft-bodied organisms and they are more expensive and complicated than water bottles. Triplicate samples should be collected from each station depth, thus allowing for statistical analysis of intrastation variability.

For small mesozooplankton (greater than 200 µm), nets are usually used (UNESCO, 1968). Additional tows may have to be made with larger nets in order to collect representative samples of larger zooplankton and larval fish. All tows should be replicated. The number of replicates necessary for the desired precision of estimation should be determined during a preliminary or pilot sampling program. A number of other considerations, including net mouth diameter, towing speed, and shipboard handling of samples, will affect sampling results. Some problems associated with the use of nets for zooplankton sampling include avoidance, which may result in underestimating abundance and diversity (McGowan and Fraundorf, 1966; Wiebe and Holland, 1968), and clogging and loss of filtration efficiency.

4.8.3 Analytical Methods Considerations

Phytoplankton

Biomass and Productivity

Phytoplankton biomass can be indirectly measured through the measurement of the concentration of chlorophyll *a* in the water. This is done through fluorometric or spectrophotometric measurements. Within these methods are differences in extraction techniques for chlorophyll determination, including various methods of filtration, solvents, temperature, and/or physical treatment (sonication or grinding) (D'Elia et al., 1986).

The use of chlorophyll *a* measurements, especially fluorometric, has become widespread primarily because the method is relatively fast, simple, and reproducible. With both the fluorometric and spectrophotometric determinations, however, the presence of accessory pigments may interfere with determination of chlorophyll *a*. Several researchers, nevertheless, have successfully used chromatographic procedures to separate interfering substances prior to determination (D'Elia et al., 1986). High Performance Liquid Chromatography (HPLC) is generally acknowledged as the most accurate (and most expensive) method for chlorophyll determinations (C.F. Zimmermann, 1990, Chesapeake Biological Laboratory, Solomons, MD, personal communication).

Other methods for estimating phytoplankton biomass used in earlier monitoring surveys include cell counts, total cell volume estimates, protein estimates, and dry weight. These and other methods have certain disadvantages related to speed of the technique or degree of accuracy (D'Elia et al., 1986).

Phytoplankton primary productivity should be measured by the ^{14}C light-dark bottle technique (UNESCO, 1973). This technique is more sensitive than, and requires shorter incubation times than the O_2 light-dark bottle method. However, the O_2 method measures gross primary production, net primary production, and respiration, using inexpensive laboratory reagents, while the ^{14}C technique estimates only net primary production and requires specialized training and equipment, as well as relatively expensive radioisotopes.

Taxonomic Analysis

Subsamples drawn from water collected in water sampling bottles should be preserved for later microscopic analysis to determine phytoplankton community composition. The choice of fixation will depend on the dominant types of phytoplankton known to inhabit a given area. (Buffered formaldehyde and Lugol's solution are two common fixatives.) Preserved phytoplankton samples normally must be concentrated for quantitative microscopic analysis. Analysis should include identification of dominant phytoplankton taxa and counts of individual species. The description and comparison of phytoplankton communities can be performed through the evaluation of species diversity, richness (number of species), evenness, or numerous other parameters. Alterations to the phytoplankton community should be analyzed in relation to other potential impacts on other biological communities. These include but are not limited to:

- Food web impacts;
- Occurrence of toxic or nuisance phytoplankton; and
- Potential secondary impacts on zooplankton or fish communities (e.g., shift from "more desirable" diatom-dominated communities to "less desirable" flagellate- or cyanobacteria-dominated communities).

Zooplankton

The taxonomic analysis should include the identification of dominant zooplankton taxa and counts of individual species whenever possible. Particular attention should be given to mesoplankton larvae of commercially, recreationally, or ecologically important species. Characteristics of the zooplankton community should include but not be limited to:

- Species composition (richness and evenness),
- Abundance,
- Dominance, and
- Diversity.

Alterations in the zooplankton community should also be analyzed in relation to potential impacts on other biological communities. Such analyses should include, but not be limited to, the structure and function of both larval and adult zooplankton communities and consideration of food web impacts.

Biological Indices

The numbers of individuals and the numbers of species have been used as indicators of anthropogenic disturbance, as well as other environmental stresses (USEPA, 1985b). Furthermore, these simple biological indices are less ambiguous and can often be as informative as diversity indices (USEPA, 1985b; Green, 1979; Hurlbert, 1971). Measures of biomass have inherent problems in the collection of the data, e.g., loss or gain of weight due to preservative medium, drying times, and evaporative weight loss.

More complicated indices such as species diversity richness, dominance, and evenness have found varying degrees of acceptance. Although diversity may not be able to pinpoint a single problematic discharge in an area that contains many, it can be an indicator of degraded water quality. Species diversity of phytoplankton (Patten, 1962) and zooplankton (Copeland, 1966; Copeland and Wohlschlag, 1968; Odum et al., 1963) communities is noted to increase with increasing distance from a pollution source. Diversity indices, which are measures of the distribution of individuals among species, have the following limitations (Green, 1984a):

- They often lack biological meaning.
- They are not robust empirical indicators of any important correlates of environmental health.
- They do not incorporate information on form and function of resident species.

- They are susceptible to biases associated with well-described taxa.

Because of this, these methods should be used in conjunction with other assessment methods to help verify results.

The dominance index is a measure of the degree to which one or a few species dominate the community. The dominance index, herein defined as the minimum number of species required to account for 75 percent of the total number of individuals, has been useful in describing community structure (Swartz et al., 1985). Its advantages are that it is easily calculated, it does not assume an underlying distribution of individuals among species, and it is statistically testable.

Indicator Species

The evaluation of the abundance of individual indicator species is generally informative and may reduce the cost of the analysis. The absence of pollution-sensitive species and the enhancement of opportunistic and pollution-tolerant species may assist in defining the spatial and temporal extent and magnitude of impacts. However, indicator variables must possess the following characteristics (Green, 1984a):

- They must provide a sufficiently precise and accurate appraisal of :
  - species of concern,
  - anthropogenic disturbances to communities, and
  - presence/absence or magnitude of anthropogenic perturbance to the ecosystem.
- They must be a cost-effective and a statistically reliable alternative to monitoring all critical measures of habitat perturbance.
- They must be appropriate for the spatial and temporal scale demanded by the study objectives.

Further studies of the response patterns of zooplankton species subjected to anthropogenic perturbations caused by discharges are required in order to select appropriate indicators of environmental impact.

4.8.4 QA/QC Considerations

Variability in measurements caused by field heterogeneity is quantitatively determined by the analysis of replicate field samples. Replicate sampling should be conducted at all field stations where measurements are to be used in comparisons. Analysis of replicate sample data is necessary for assessing the reliability of such comparisons.

Laboratory performance and calibration should be verified at the beginning and periodically during the time analyses are performed. Commercially available chlorophyll is available and recommended for use in calibration. Chlorophyll quality control samples are available from EPA's Environmental Monitoring and Support Laboratory in Cincinnati, Ohio. Blind, split, or other control samples can be used to evaluate performance. The *Interim Guidance on Quality Assurance/Quality Control (QA/QC) for the Estuarine Field and Laboratory Methods* (USEPA, 1985b) provides a standard operating procedure for chlorophyll measurements.

4.8.5 Statistical Design Considerations

Temporal stratification of the data should not be attempted until sufficient knowledge of long-term natural cycles is attained. Initially, simple regression analyses may be conducted on seasonally stratified data to identify monotronic temporal trends. Further examinations of whether conditions are improving or degrading over time may be conducted using statistical time series analyses (e.g., temporal autocorrelation, spectral analyses, etc.).

Information derived from water quality monitoring will be important in interpreting the results of plankton sampling. For example, phosphate concentrations may indicate the cause of a phytoplankton "bloom," while measures of dissolved oxygen levels may describe the effects of the "bloom" on other living estuarine resources. Therefore, the selection of water quality and plankton sampling strategies must not be done independently. These programs should be integrated to the fullest extent possible to allow correlation of observed responses to changes in water quality parameters. Also, alterations to the plankton community should be analyzed in relation to other impacts on biological resources such as food web impacts on fish communities.

4.8.6 Use of Data

The analyzed, collected data will be used initially to identify the prominent species and relate the temporal and spatial distribution patterns to water quality parameters. Statistical techniques will be used to make these comparisons. As the discharge guidelines are implemented, a plankton monitoring program can be used to track the effectiveness of maintaining nutrient levels. As plankton levels fluctuate, their effects on oxygen levels and fish and shellfish communities can be assessed. Plankton monitoring strategies should be able to delineate between natural variability in plankton stocks and variations caused by anthropogenic changes in nutrient concentrations.

Characterization of phytoplankton species abundance, distribution, and primary productivity provides indications of water quality conditions. Monitoring changes in phytoplankton community composition and densities is critical for the interpretation and evaluation of long-term trends in water and habitat quality. Further understanding of the causes of excessive water column and sediment oxygen demand requires tracking of

photosynthetic activity and metabolic rates over time (Chesapeake Executive Council, 1988b). Zooplankton abundance and distribution are affected by both changes in phytoplankton and changes in predator populations. Therefore, population characteristics of this group can indicate symptoms of water quality problems, fishing pressure, and other habitat problems for predator species (Chesapeake Executive Council, 1988b).

4.8.7 Summary and Recommendations

Rationale

- Track phytoplankton and herbivore populations if changes in trophic structure are suspected.
- Purpose of monitoring is to assess the effectiveness of the management programs in mitigating the potential impacts caused by excessive nutrient inputs and resulting changes in the planktonic community biomass and structure/function.

Monitoring Design Considerations

- Collect both phytoplankton and zooplankton samples at specific depths throughout the water column.
- Phytoplankton samples should be taken with water bottles and pumps used for chlorophyll concentrations.
- Zooplankton samples should be taken with bottles or nets of varying sizes.
- Monitoring programs should consider natural and temporal fluctuations in plankton biomass and species composition.
- Other components of the overall monitoring program, including water quality monitoring and fish and shellfish communities, should be analyzed relative to plankton community data to establish relationships and trends.
- Selected biological indices should retain biological meaning, be robust indicators of marine "health," and incorporate species form and function.
- Indicator species should possess the following characteristics:
  - sensitive to planktonic perturbances of concern;
  - cost-effective and statistically reliable alternative to measuring all species in a monitoring program;
  - statistically reliable indicative measures of habitat perturbance; and
  - appropriate for the spatial and temporal scale determined by the study objectives.

- Selection of reference sites is key to the evaluation of environmental impact due to anthropogenic perturbances; several reference sites may be requried to provide proper control for sampling sites.

Analytical Methods

- It is recommended that consistent types of sampling gear and location and timing of sample collection be implemented to allow for comparisons between studies.
- Phytoplankton biomass can be determined using fluorometric or spectrophotometric methods using a variety of filtration and extraction techniques.
- Phytoplankton productivity should be measured using the ^{14}C light-dark bottle technique.
- Taxonomic analysis should include identification and counts of dominant species.

QA/QC Analysis

- Replicate samples should be taken at all field stations where applicable.
- Laboratory performance evaluations and calibrations must be done on a regularly scheduled basis.
- Standard chlorophyll samples, obtained from EPA, should be used for calibrations.

Statistical Design Considerations

- Power analyses may be applied to determine the appropriate number of sample replicates required to detect a specified difference.
- Temporal integration of the data should not be attempted until sufficient knowledge of long-term natural cycles is attained.

Data Use

- Identify dominant species.
- Detect short- and long-term spatial and temporal trends in overall biomass and productivity.
- Detect short- and long-term spatial and temporal trends in species abundance, distribution, and composition.
- Examine relationship between water quality condition and trends in plankton community characteristics.

- Examine relationship between plankton community characteristics and impacts on other living resources (e.g., fish and shellfish communities).

4.9 HABITAT IDENTIFICATION METHODS

Sensitive marine habitats serve a vital purpose as spawning grounds, nursery grounds, forage areas, energy sources, shelter, and migratory routes and destinations for a multitude of marine organisms. Pollution-related damage can cause long-lasting harm to these habitats to the extent of altering ecosystem diversity and function.

For the purpose of this discussion, a sensitive habitat can be defined as a physical area used, at least for a significant portion of the year, by a disproportionate abundance of individuals and/or species, or as an area essential to the functioning of the ecosystem.

Habitats need not be permanent entities but may expand and contract on a seasonal basis. Examples of transitory habitats include sea grass beds and ice floes. Marine habitats can be organized in a hierarchical structure to be considered under the discharge guidelines and can be grouped in the following manner:

Subtidal
- Rock bottom
- Bedrock/rubble
- Coral
- Oyster/clam shell reef
- Unconsolidated bottom
- Cobble/gravel
- Sand
- Mud-rich organic
- Submerged aquatic vegetation (SAV)
- Sea grass (*Zostera, Thalassia*)
- Algal and other submerged plant communities
- Water column

Intertidal
- Emergent vegetation (along exposed coasts)
- Marshes
- Mangroves

Ice Floes

4.9.1 Rationale

The ocean discharge guidelines that relate to the presence of and impact on marine habitats are the following;

- Importance of the receiving water area to the surrounding biological community, e.g., spawning sites, nursery/forage areas, migratory pathways and areas necessary for critical life stages/functions of an organism;
- The existence of special aquatic sites, including (but not limited to) marine sanctuaries/refuges, parks, monuments, national seashores, wilderness areas, coral reefs/seagrass beds;
- Existing or potential recreational and commercial fishing; and
- Any applicable requirements of an approved CZMP.

The identification and delineation of these habitats should be made in both temporal and spatial dimensions to account for variations in use by the biological community throughout the year. Both the aerial extent and the functional value to biological resources must be determined in considering the identification and importance of a sensitive marine habitat.

Even though an effluent discharge may not directly alter the biological population in a certain area, if the habitat essential for the survival of those individuals is destroyed, the ecological results could be the same as if the population had been exposed directly. The loss of critical habitat could cause the supported population to be either removed or displaced to less suitable habitat.

Monitoring of discharge effects can help to ensure that long-term sublethal doses do not accumulate and become toxic. It can also provide assurance that effects such as increased turbidity or sedimentation do not reduce biological resource productivity below sustainable levels.

During the permit review process, all important and sensitive habitats should be identified and delineated to serve as a baseline for future monitoring activities. Habitat quality in terms of functional values for fish, bird, and marine mammal populations must also be taken into account to gain a complete valuation of the resources that could be affected by discharges. Some habitat monitoring activities overlap with other biological monitoring activities described in other sections. For habitats, the main concern is to map the physical boundaries and to correlate biological populations to changes in habitat distributions and functional values.

4.9.2 Monitoring Design Considerations

Habitat monitoring is a two-part process. First, the aerial coverage and boundaries must be determined; second, the importance or the functional value to biological resources must be determined. The first determination can be made by using charts, remote sensing, or submersible remotely operated vehicles. The second analysis is carried out by using quantification techniques to establish the value of that habitat for particular

species. The combining of these two techniques allows the identification of those habitats critical to the functioning of the ecosystem. The determination of the functional value of a habitat is much more subjective than delineation and must be done with care. The results of these procedures should be combined with data on trends in species distribution and water quality to provide a more complete assessment of the causes and potential effects of habitat degradation. Habitat monitoring will also indicate which environmental parameters and areas are critical to the ecosystem so that monitoring activities can be tailored to assess these needs.

To conduct a habitat trend analysis, the sampling design must account for natural variations caused by extraordinary meteorological events and other phenomena. The recovery rate of habitat functions and its effects on the functional analysis described above must also be considered. The primary concern of the habitat assessment is to accumulate an adequate set of baseline data from which trends can be established. During this initial phase, seasonal variations must be taken into account during sampling phases. Sampling and delineation should be carried out at times of maximum biomass or physical extent. For example, SAV beds or ice floes go through seasonal declines (or disappearances) and thus a finite period of time exists to conduct sampling. Diurnal factors such as tides and weather conditions also affect biological populations, and sampling must be conducted taking these factors into account.

4.9.3 Analytical Methods Considerations

Habitats are generally classified in a hierarchical scheme as mentioned above. Classification methods that can be used are referenced in Appendix A. The first course of action in identifying, delineating, and evaluating critical marine habitats entails mapping out the boundaries of the habitat, while the second involves a determination of its value as an ecological resource. Table 4-23 lists the methods that can be used to identify and evaluate sensitive habitats.

Table 4-23. List of Analytical Methods

Areal Trends	Functional Trends
Aerial Photography	Habitat Evaluation Procedure (HEP)
Maps	Habitat Suitability Modeling
Satellite Imagery	Minimum Habitat Matrix
Remotely Operated Vehicles	Wetland Evaluation Technique (WET)

Habitat Delineation

Aerial Photography

Various government agencies (NOAA and USGS among others) have used aircraft to produce aerial photographs of wide areas for a number of years. The use of photographs in delineating marine habitats is of limited value since many features below the low tide line cannot be viewed. Aerial photographs can be produced in a large range of scales and spatial resolutions depending on the area in question. Fine detail can be resolved for relatively small tracts, while large areas can be viewed at lower resolutions to define gross features. In some regions there is an extensive record of historical photographs that can be used to spot trends and to establish a baseline. New overflights can be expensive and require ideal weather conditions, but they can be of value for intertidal mapping and for mapping some submerged aquatic vegetation and other shallow-water habitats.

Maps

As a preliminary method, maps and charts can be used to obtain a quick assessment of the presence and delineation of sensitive habitats in the discharge area. Maps are the least expensive spatial data source and are also the most simple. Nautical charts, compiled by the Coast and Geodetic Survey of NOAA, contain bottom characteristic information that could indicate the location of areas of biological importance. This information is generally accurate for shallow-water areas but may be fragmentary or nonexistent for areas farther offshore. The coordinated use of U.S. Geological Survey topographic quadrangle maps, U.S. Fish and Wildlife Service National Wetlands Inventory maps, and NOAA nautical charts can serve as an information base.

The principal advantages of using maps for delineating habitats is their ease of use and their low cost. Disadvantages include the inaccuracies in the plotting of habitat boundaries and the fact that the maps in use may be outdated and thus do not reflect current conditions. In locations that have a history of human use, there may be an extensive list of earlier chart or map editions. These earlier editions can be an excellent resource for spotting changes in habitat structure.

Satellite Imagery

Satellite imagery is a potential source of data when very large study areas are evaluated and fine spatial detail is not required. This technique is useful to a maximum depth of approximately 30 meters, and habitats can be resolved only to a minimum of roughly 30 to 50 square meters. For large study areas, the cost of satellite images can be high, especially when a long-term temporal series may be required for monitoring purposes. If the area under investigation contains large homogenous habitats, the resolution size may not be a constraining factor. A major benefit of these images is that the spectral

properties and brightness values are recorded digitally. This technology lends itself to advanced spatial analysis methods that can be used to quantify productivity values. These determinations are not possible with most aerial photographs.

Remotely Operated Vehicles

Remotely operated vehicles (ROVs) are small, unmanned, remotely operated submersibles that are equipped with lights and cameras used to view portions of the ocean bottom. These submersibles receive power and commands from a support vessel and are highly versatile for identifying possible habitats. EPA Region 4 has used ROVs to investigate and record the ocean bottom immediately surrounding the discharge outfalls of oil and gas drilling wells. This methodology produces a precise, nondestructive record of the nature and extent of any physical habitat. The main liability of this system is that unless vast amounts of time are spent, only small areas can be investigated. These systems are also rather expensive and require well-trained technicians to operate them. In addition, a ROV is limited by the length of its umbilical and by the visibility of the water column in the search area. The visibility problem may be compensated for by the addition of such technology as side scan sonar or sonar attached to the ROV. Because of these constraints, the use of this system would be confined to areas of most concern near the zone of initial dilution.

Functional Analysis

Functional analysis attempts to assess the use of the habitat by biological resources and to compare the value of one habitat to that of another. Another aspect of functional analysis is to calculate the change in a habitat's value over time. These processes entail site-specific studies that can involve a large amount of time and money. The data required by this technique can be collected in connection with the techniques described in the biological methods section of this report. The analysis of these data will center on spatial and temporal variations at a particular habitat. Various methods for conducting functional analysis are referenced in Appendix A. Some of the most widely used procedures are described below.

Habitat Evaluation Procedures

Habitat evaluation procedures (HEPs) are procedures developed by the U. S. Fish and Wildlife Service to document the quality and quantity of available habitat for selected wildlife species. The data generated are used to compare two habitats at the same point in time and a single habitat over a length of time. By combining these two sets of information, the impact of proposed discharges can be quantified (Lonard and Clairain, 1986).

In the evaluation procedure, the habitat quality of selected species is documented based on an evaluation of the ability of key habitat components to supply the life requisites of the selected species. The evaluation involves using the same key habitat components to compare existing habitat conditions and the optimum conditions for the species of interest (USFWS, 1980).

The limitations in using this approach involve the use of habitat quality as an evaluation parameter, which limits the methodology to those situations in which measurable and predictable habitat changes are an important variable. This approach also forces a long-term averaging type of analysis. There is no assurance that populations will exist at the levels predicted by the analysis since all the environmental or behavior variables that affect population levels may not be included. In addition, socioeconomic or political constraints not taken into account may prevent the actual populations from reaching their predicted levels (USFWS, 1980).

Habitat Suitability Modeling

Habitat suitability modeling consolidates habitat use information into a framework appropriate for field application and is scaled to produce an index between 0.0 (unsuitable habitat) and 1.0 (optimum habitat). Habitat suitability models are designed for use with the habitat evaluation procedures described above (Toole et al., 1987). Multiple regression equations are used to describe relationships between environmental characteristics and productivity. Once the model has been validated, sensitivity analysis can be carried out to project the results of varied discharge rates on the health of the site.

For modeling to make reliable predications, the input data must accurately reflect the range of natural conditions observed. This may involve expensive and time-consuming field studies in which the error limits and the QA/QC parameters must be well defined. The reliability and the extent of the field data can be a major limitation in these studies.

Minimum Habitat Matrix

Minimum habitat guidelines for various species are a technique developed with the ultimate goal of reestablishing balanced ecosystems in environmentally stressed areas. This method is designed to provide information on the minimum habitat quality needed by a target species and identifies those factors (both environmental and ecological) required for the species. This information is formatted into a habitat requirement matrix that defines the habitat parameters needed for successful reproduction and survival of the indicated species. Such matrices can indicate the vital environmental parameters that should be monitored, thus facilitating the development of monitoring programs. This process is used to estimate the feasibility, benefits, and potential costs of maintaining and protecting an estuarine environment suitable for the successful reproduction and survival of the indicated species (Chesapeake Executive Council, 1988a).

One potential problem with this method is that the primary indicated species and those organisms on which the target species depend for food are both tracked with the intention of maintaining habitat quality for both. This method may not completely recognize the complex species interdependence within marine environments.

Wetland Evaluation Technique

The wetland evaluation technique (WET) was initially developed by the Federal Highway Administration and revised by the U.S. Army Corps of Engineers to assess the function and value of various components of habitats and their suitability for specific fish and invertebrate species. WET evaluates functions and values in terms of social significance, effectiveness, and opportunity (Adamus et al., 1987). Social significance assesses the value of a site to society due to its special designation, potential economic value, and strategic location. Effectiveness assesses the capability of a wetland to perform a function as a result of its physical, chemical, or biological characteristics. Opportunity assesses the opportunity for a habitat to perform a function to its level of capability.

WET assesses functions and values by characterizing a habitat in terms of its physical, chemical, and biological processes and attributes (Adamus et al., 1987). This characterization is accomplished by identifying threshold values for predictors. Predictors are simple, or integrated, variables that directly or indirectly measure the physical, chemical, and biological processes or attributes of a habitat and its surroundings.

4.9.4 QA/QC Considerations

Since sensitive habitat identification is relatively subjective, close controls must be exercised during sampling and delineation. In the delineation of habitats, two individuals should independently define the boundaries of a single habitat area. Any disagreement between the two should be rectified before a final determination is reached. Sampling programs demand a high level of expertise on the part of the individuals conducting them. All personnel must have an advanced understanding of such topics as taxonomy, sampling techniques, and statistics. The sampling strategy used will depend at least in part on the level of expertise or training available. The selection of a functional analysis method should be made after a review of the strengths and limitations of each method has been conducted.

4.9.5 Statistical Design Considerations

In designing a habitat monitoring program, the researcher must first determine the resources available. This will determine the extent of the monitoring program and the level of detail it can provide. Aerial assessment can be done with limited resources, while functional analysis requires greater commitments of both finances and time.

Functional assessments require that the researcher decide which functions are of greatest interest in the area of study or which will provide the most information concerning the habitat attributes perceived to be of greatest value to the area.

Most habitat evaluation techniques involve some form of ranking, either quantitative or qualitative. The purpose of the monitoring program is to periodically compare baseline conditions to conditions after the permit has been issued. In terms of habitat monitoring, this means comparing the baseline acreage or functional rating of the habitat to conditions some time after discharge has commenced. Therefore, the ability to replicate results is imperative.

4.9.6 Use of Data

The object of the data set is to obtain a clear and precise spatial and temporal picture of the physical extent of a sensitive habitat. An evaluation of the functional value of that habitat is also made so that the effects of various impacts can be judged. Sensitive habitats should be clearly delineated on nautical charts or topographic maps along with a complete description of their composition. The description should include a characterization of the extent of natural seasonal variations in the boundaries. A complete description of the functional value of the habitat to the biological community, as well as an estimate of potential for loss of the resource from the effects of effluent discharge, should also be made.

These data will act as a baseline for future monitoring programs that will track the changes to the system. The baseline study and the monitoring program should be able to discriminate between natural variations and discharge-induced changes.

4.9.7 Summary and Recommendations

<u>Rationale</u>

- A sensitive habitat can be defined as a physical area utilized, at least for a significant portion of the year, by a disproportionate abundance of individuals and/or species, or as an area essential to the functioning of the ecosystem.
- Sensitive marine habitats should be identified and their boundaries delineated as part of an ongoing effort to assess the potential for impacts from effluent discharges on ecological communities.
- Monitoring programs that assess both the physical boundaries of the habitats and the support functions that they serve for biological communities should be conducted when sensitive habitats are present.
- Damage to habitats from discharge effluents can cause irreversible harm and create severe changes in ecosystem function and diversity.

- Monitoring programs can provide assurance that effects such as increased turbidity or sedimentation do not reduce biological resource productivity below sustainable levels.

Monitoring Design Considerations

- Habitat monitoring consists of two parts: (1) aerial coverage and boundary delineation and (2) functional analysis, which determines the value of the habitat to the system.
- Habitat delineation can be fairly straightforward, but functional analysis is rather subjective and must be done with care.
- Sampling strategies must account for variations caused by natural processes and must be done at times of maximum extent or biomass.
- A functional analysis technique should be selected after a review of the strengths and limitations of each method for each particular site.

Analytical Methods Considerations

- Sensitive habitats can be identified using maps, charts, and photographic techniques. ROVs can be used to investigate near-field habitats in the zone of initial dilution.
- Functional analysis determines the importance of a habitat to the biological community.
- Functional analysis techniques are based on theoretical relationships of the ecosystem; consequently, both the theory and the data used should be vigorously evaluated.
- For habitat identification and delineations, the following techniques can be used:
  - aerial photography,
  - maps/charts,
  - satellite imagery, and
  - remotely operated vehicles.
- Functional analysis can be carried out using various methods. Those described are the following:
  - habitat evaluation procedures (HEP),
  - wetland evaluation technique (WET),
  - habitat suitability modeling, and
  - minimum habitat matrix.

QA/QC Considerations

- Personnel identifying habitat should have advanced training, and two people should independently define the boundaries.

Study Design Considerations

- Ability to replicate results is important.
- The extent of the monitoring program can be based on funding level.
- Aerial assessments are inexpensive, while functional analyses are more expensive and more labor-intensive.

Use of Data

- Data collected will consist of charts identifying the boundaries of sensitive habitats accompanied by complete descriptions.
- Functional analysis results will indicate which habitats are critical and the effects observed under various discharge scenarios.

4.10 BIOACCUMULATION

Bioaccumulation is the overall process of biological uptake and retention of chemical contaminants obtained from foods, water, sediments, or any combination of exposure pathways. A number of expressions are used to describe the bioaccumulation potential of contaminants. These terms are explained in Table 4-24. Bioaccumulation is a consequence of an organism's physiological limitations to transform and excrete the invading chemical substances. Potential consequences of bioaccumulation of chemical contaminants in marine organisms include, but are not limited to:

- Significant mortality to susceptible organisms;
- Lethal or sublethal chronic toxic responses at later stages of the life cycle or under conditions of stress for susceptible organisms; and
- Transference of increasingly greater concentrations of toxic pollutants to organisms at higher trophic levels, including humans.

Bioaccumulation analyses provide the link between exposure and effects and thus can generate important insights into ecological effects, human health risks, and routes and extent of pollutant exposure.

4.10.1 Rationale

The Clean Water Act section 403 point source discharge program requires (among other things) a determination of:

Table 4-24. List of Terms

Bioavailability	A measure of the physicochemical access that a toxicant has to the biological processes of an organism. The less the bioavailability of a toxicant, the less its toxic effect on an organism (USEPA, 1991c).
Bioaccumulation	Uptake and retention of substances by an organism from its surrounding medium and from food (USEPA, 1990a).
Bioconcentration	Uptake of substances by an organism from the surrounding medium through gill membranes or other external body surfaces (USEPA, 1990a).
Biomagnification	The process by which the concentration of a compound increases in different organisms occupying successive trophic levels (USEPA, 1990a)

- The quantities, composition, and potential for bioaccumulation or persistence of the pollutants to be discharged;
- The potential transport of the pollutants by biological, physical, or chemical processes;
- The composition and vulnerability of potentially exposed biological communities; and
- Potential direct or indirect impacts on human health.

Each of these determinations is addressed to some degree through bioaccumulation studies.

Monitoring of bioaccumulation data is essential in relating the presence of selected chemical residues in marine waters and sediment to their transfer and accumulation in marine organisms and potential transfer to humans. Toxics can occur in the water column at or near analytical detection limits; however, over time, these contaminants can accumulate in fish and shellfish tissue to measurable concentrations. In areas where water and sediments show measurable contaminant levels, it is difficult at best to predict rates of uptake and bioaccumulation, although direct monitoring can provide spatial and temporal records of the concentration and bioaccumulation of toxics. The assessment of bioaccumulation in the surrounding biological community should be a component of a monitoring program required in a permit issued under the "no irreparable harm" clause of section 403.

4.10.2 Monitoring Design Considerations

Typically, bioaccumulation studies are formulated according to the study objectives. If the objective is to determine the effects of bioaccumulation on human health, then commercial and/or recreational fish and shellfish are tested. If determining the effects on the habitat or ecosystem is the objective, usually the benthic macroinvertebrates are surveyed. Unfortunately, comparisons between studies are valid only if the study designs and procedures are comparable. Standardization of monitoring design would allow for comparison between various studies.

A common deficiency in many programs is the inability to collect sufficient tissue biomass of appropriate species across sampling locations throughout the study. The selection of appropriate species and tissues must account for natural fluctuations in populations as well as changes due to anthropogenic perturbations. Indigenous species initially present may not be available later, limiting temporal and spatial comparisons.

Selection of Target Species

A key component of any bioaccumulation monitoring program is the selection of target species. Concentrations of chemical residues in tissues of target species serve as indicators of contamination throughout the biological system. The fundamental criterion is the ability to use the selected species to make comparisons between sampling locations and sampling periods.

Target Species Characteristics

It is recommended that the target species possess the following characteristics (USEPA, 1985a):

- High bioaccumulation potential for selected contaminants of concern;
- Weakness or absence of metabolic regulation of selected contaminants to allow assessment of a "worst-case scenario";
- Abundant enough, temporally and spatially, to allow for adequate sampling;
- Large enough to provide adequate amounts of tissue for analysis;
- Sessile or sedentary in nature to ensure that bioaccumulation is representative of the study area;
- Easily collected; and
- Part of an existing data base of exposures and sensitivity.

Suggested fish and macroinvertebrate target species for various regions of the United States are listed in Tables 4-25 and 4-26 (USEPA, 1985a).

Secondary considerations based on economic importance and status as a bioassay organism may be applied to further winnow the list of candidate target species to a practical number of species to be analyzed.

Candidate target species should be objectively ranked based on ecological characteristics that would enhance their potential for bioaccumulation and facilitate sampling and analytical procedures (USEPA, 1985a). The advantages of using benthic macroinvertebrates as target species are:

- They are sendentary and therefore represent bioaccumulation in the study area.
- They represent a significant food source for higher trophic levels and therefore indicate the existence of contaminants in the food web.

Table 4-25. Highest-Ranking Candidate Fish for Use as Bioaccumulation Monitoring Species

State	Locality	Species	Secondary Selection Criteria: Economic Importance	Secondary Selection Criteria: Bioassay Species
MASSACHUSETTS	Swampscott	Winter flounder	Yes	Yes
		Yellowtail flounder	Yes	Yes
		Ocean pout	No	No
		Windowpane	No	No
	Lynn	Winter flounder	Yes	Yes
		Yellowtail flounder	Yes	No
		Ocean pout	No	No
	South Essex	Winter flounder	Yes	Yes
		Yellowtail flounder	No	No
		Windowpane	No	No
		American eel	No	No
		Ocean pout	No	No
	Boston	Winter flounder	Yes	Yes
		Yellowtail flounder	Yes	No
		Ocean pout	No	No
		Windowpane	No	No
	Fall River	Winter flounder	Yes	Yes
		Windowpane	No	No
	New Bedford	Winter flounder	Yes	Yes
		Scup	No	No
		Summer flounder	Yes	Yes
RHODE ISLAND	Newport	Winter flounder	Yes	Yes
		Scup	Yes	No
		Weakfish	Yes	No
NEW YORK	Upper East River	Winter flounder	Yes	Yes
		Windowpane	No	No
		Weakfish	Yes	No
	Lower East River	Spot	Yes	Yes
		Scup	Yes	No
		American eel	No	No
	Lower Hudson River	Hogchoker	No	No
NEW JERSEY	Cape May	Spot	Yes	Yes
		Red hake	No	No
		Windowpane	No	No
		Summer flounder	Yes	Yes
VIRGINIA	Portsmouth	Spot	Yes	Yes
		Summer flounder	Yes	Yes
		Atlantic croaker	No	No
		Hogchoker	No	No
	Virginia Beach	Spot	Yes	Yes
		Red hake	No	No
		Summer flounder	Yes	Yes

Table 4-25. (continued)

State	Locality	Species	Secondary Selection Criteria: Economic Importance	Secondary Selection Criteria: Bioassay Species
CALIFORNIA (Northern)	San Francisco	English sole	Yes	Yes
		Pacific sanddab	Yes	No
		Big skate	No	No
	Oakland	English sole	Yes	Yes
		Starry flounder	Yes	No
		Pacific staghorn sculpin	No	No
	Monterey	English sole	Yes	Yes
		Curlfin sole	Yes	No
	Santa Cruz	English sole	Yes	Yes
	Watsonville	English sole	Yes	Yes
		Curlfin sole	Yes	No
CALIFORNIA (Southern)	Goleta	Dover sole	Yes	No
		Pacific sanddab	Yes	No
		Longspine combfish	No	No
		Spotted cusk-eel	No	No
	Santa Barbara	English sole	Yes	Yes
		Pacific sanddab	Yes	No
	L.A. County	Dover sole	No	No
		Curlfin sole	No	No
		English sole	Yes	Yes
	Orange County	Dover sole	Yes	Yes
		Pacific sanddab	Yes	Yes
		English sole	Yes	Yes
	Hyperion	Dover sole	Yes	No
	Oceanside	Longspine combfish	No	No
		Big skate	No	No
	Escondido	California skate	No	No
	San Elijo	Dover sole	Yes	No
		Blackbelly eelpout	No	No
		Pacific sanddab	Yes	No
		English sole	Yes	Yes
	San Diego	Dover sole	Yes	No
		English sole	Yes	Yes
		Pacific sanddab	Yes	No
		Longspine combfish	No	No
WASHINGTON	Central Puget Sound	English sole	Yes	Yes
		Dover sole	Yes	No
		Rock sole	Yes	No
		Spotted ratfish	No	No
		Rex sole	Yes	No
		C-O sole	Yes	No

Table 4-26. Recommended Large Macroinvertebrate Species for Bioaccumulation Monitoring

Region	Recommended Species[a]
Massachusetts to Virginia	American lobster (*Homarus americanus*) Eastern rock crab (*Cancer irroratus*) Hard clam (*Mercenaria mercenaris*) Soft-shell clam (*Mya arenaris*) Ocean quahog (*Artica islandica*) Surf clam (*Spisula solidissima*) Edible mussel (*Mytilus edulis*)
Alaska to California	Spiny lobster (*Panulirus interruptus*) Dungeness crab (*Cancer magister*) Rock crab (*Cancer antennarius*) Yellow crab (*Cancer anthonyi*) Red crab (*Cancer productus*) California mussel (*Mytilus californianus*)
Florida, Virgin Islands, and Puerto Rico	Spiny lobster (*Panulirus argus*)
Hawaii	Spiny lobster (*Panulirus penicillatus*)

[a] Additional species that may occur at specific discharge sites and are considered acceptable bioaccumulation monitoring species include the American oyster (*Crassostrea virginica*) and the Pacific oyster (*Crassostrea gigas*).

A disadvantage of macroinvertebrates is the lack of adequate tissue biomass for analyses. Adult fish are often used not only for their significant biomass, but also as a measure of possible contaminants available to the human population. Fish, however, are motile and may not be representative of the study area.

Ideally, studies should include samples of numerous species from many phyla. This diversity will ensure the collection of bioaccumulation data for a wide spectrum of contaminants and will indicate a species' potential to bioaccumulate those contaminants. For example, oysters and other bivalves are ideal for monitoring bioaccumulation of PAHs because of their limited ability to metabolically transform these contaminants. The monitoring of a food chain could establish the potential for transport from one trophic level to another. Species with extensive bioaccumulation data would be preferred. A fundamental criterion is the ability to use the selected species to make comparisons between sampling locations and sampling periods.

Caged Indicator vs. Indigenous Species

The California Mussel Watch Program, the U.S. Mussel Watch Program, and the NOAA Status and Trends Program have employed the use of both resident and caged transplant mussels to monitor bioaccumulation of toxic chemicals over space and time (Goldberg et al., 1978; Boehm, 1984; Ladd et al., 1984). Caged indicator target species offer several advantages over indigenous species :

- The biology and ecology of these indicator species are usually well described.
- Descriptions of culture and/or maintenance of the organisms under laboratory conditions are often available.
- The method provides control of initial temporal and spatial variation of individuals and/or biomass.
- The method allows the use of a specific age, size, and/or genetic stock.
- The method ensures on-site bioaccumulation.

By controlling for initial conditions, the rate of change of tissue contamination may be calculated. In addition, the Long Island Sound National Estuary Program has shown that performing chemical residue analyses on caged indicator species appears to be a promising approach for identifying sources of pollution (USEPA, 1982c).

The use of a caged indicator species indicates only uptake due to exposure to the water column, and unfortunately methods for caging sediment-ingesting organisms are not yet available. The bioaccumulation of sediment-sorbed contaminants is thought to be indicated by transfer through interstitial waters (Knezovich and Harrison, 1987), and the results often do not relate to species found on site. Therefore, caged organisms may not provide a full-spectrum profile of the contaminants that are bioavailable at any one site.

The advantages of indigenous species are as follows:

- Results obtained will relate directly to those species which may be impacted and will provide a direct measure of potential risks to human health.
- There is no cost of transplanting organisms.
- There is no possibility of loss of cage/buoy system.
- There is no possibility of introduction of a "nuisance" species.

Unfortunately, common indigenous species may not meet the criteria for use as bioaccumulation target species, negating any advantage of using a native species. The most common problem is collecting sufficient tissue biomass of appropriate species

across sampling locations throughout the study. The composition of benthic community species is subject to large natural fluctuations, as well as fluctuations in response to pollution (Pearson and Rosenberg, 1978); indigenous species initially present may not be available later, limiting temporal and spatial comparisons.

Selection of Tissues

The type and location of tissue analyzed will depend on the objectives of individual monitoring programs. It is strongly recommended that all analyses be conducted with the same species and tissue type in order that scientifically and statistically valid comparisons may be made.

In fish, liver tissue is closely associated with regulation and storage of many toxic substances (Fowler, 1982). Its high fatty tissue content tends to accumulate lipophilic contaminants. Therefore, contaminant levels in the liver can be used to estimate the range of contaminants being assimilated. For macroinvertebrates, hepatopancreas or digestive gland tissue performs functions analogous to those of fish liver tissue.

Contaminants in edible muscle tissue represent those contaminants that are retained in a form that allows transfer to humans. Sampling of muscle tissue is appropriate for human exposure assessments and quantitative health risk determinations. Within a fillet, contaminant concentrations may vary; therefore, it is recommended that a consistent location within the muscle tissue be analyzed (USEPA, 1989a).

Whole-body analyses should be conducted when predators consume the whole body of the target organism. If organisms are not cleansed of materials contained in the digestive tract, contaminants in the gut contents will be included in the analyses. To provide the most accurate estimate of the total amount of contaminants available to most macroinvertebrate predators, this type of cleansing may not be required.

Total Organic Carbon and Acid Volatile Sulfides, and Tissue Lipid Normalization

Toxic sediment concentrations of hydrophobic contaminants have been found to be related to the total organic carbon content (TOC) of the sediment (Karickhoff et al., 1979). The bioavailability of metal contaminants has been found to be related to the acid volatile sulfide (AVS) concentrations of the sediment (DiToro et al., in press). TOC and AVS normalizations have been conducted to estimate the concentrations of sediment contaminants that are bioavailable over different sampling locations. Sediment TOC and AVS normalizations are recommended to account for the variability in bioavailable sediment contaminant concentrations between locations.

Lipids appear to be a storage site of organochlorines, hydrocarbons, and other hydrophobic contaminants in a variety of organisms (de Boer, 1988). In fact, just as TOC and AVS are considered by many to be the most important parameters in defining

organic and metal concentrations in sediments, lipid content is considered the salient parameter in defining hydrophobic residue concentrations found in tissues (Phillips and Segar, 1986). Likewise, tissue lipid normalizations have been suggested to account for the variability in tissue contaminant concentrations between individuals.

Normalization of sediment contaminant concentrations to TOC and AVS and normalization of tissue contaminant concentrations to the tissue lipid concentrations have been made to permit comparisons of toxic chemical residues in tissues between studies, locations, individuals, and tissue types. These sediment and tissue normalizations assume the following: :

- Contaminants partition predominantly to sediment TOC, sediment AVS, and organismal lipids.
- Contaminants partition reversibly between the sediment particles and the organism.
- Rapid steady-state kinetics of contaminants are maintained.
- Sediment is the only source for the bioaccumulation.

Lipid normalization has been performed in order that individuals with differing body fat levels may be compared. Again, it is essential that all analyses be conducted with the same tissue type from the same species to ensure that scientifically and statistically valid comparisons may be made. TOC/lipid normalized accumulation factors (AF) have also been used to predict tissue residue concentrations (Ferraro et al., 1990; Lake et al., 1987).

Threefold differences in lipid concentration may result as a consequence of various lipid analysis techniques. Development of protocol standardization or intercalibration between lipid techniques is required before chemical residues in tissues can be compared between studies. Microtechniques have been developed for analyses of lipids from single individuals (Gardner et al., 1985). Finally, the decision to normalize for TOC, AVS, and percent lipid will depend on monitoring program objectives. For example, if the monitoring objective is to identify "hot spots"—i.e., those areas where high body burdens present a risk to human and ecological health—normalization may not be appropriate. If the objective is to identify temporal trends in bioaccumulation rates, however, normalization may be justified.

Selection of Sampling Period

The timing of sampling should be based on biological cycles that influence an organism's susceptibility to bioaccumulation. For example, for crustaceans, just after molting and before hardening of the integument occurs, restricting its permeability, there is a significant increase in potential for bioaccumulation of toxic contaminants. The

frequency of sampling should be related to the expected rate of change in tissue concentrations of contaminants. A consistent sampling period is recommended in order that spatial and temporal comparisons may be conducted.

For many aquatic vertebrate and invertebrate organisms, the reproductive cycle exerts a major influence on tissue concentrations of many contaminants, especially lipophilic compounds (Phillips, 1980). For many species, lipophilic contaminants are transferred from the muscle and liver to the eggs as lipids are mobilized and transported to the egg during oogenesis (Spies et al., 1988; Gardner et al., 1985). There is evidence that lipophilic contaminants have deleterious effects on the developing egg and/or the larvae (Spies et al., 1988; Hansen et al., 1985; Niimi, 1983). Transfer of contaminants from adult to egg and its potential impacts on future fisheries recruitment and fisheries production are under investigation.

It is recommended that target species be sampled when tissue contaminant concentrations are expected to be at their highest levels in order to evaluate worst-case scenarios. For those organisms where the body is consumed whole, contaminant levels are usually at their highest at or just before spawning. For organisms where the muscle tissue is consumed, contaminants in muscle tissue usually reach a peak well before spawning.

4.10.3 Analytical Methods Considerations

Factors to be considered during the choice of an appropriate analytical method include the parameters of interest, desired detection limits, sample size requirements or restrictions, methods of preservation, technical and practical holding times, and matrix interferences. Several USEPA documents (1986b, 1987c, 1990c) discuss the common analytical problems encountered during monitoring analyses of tissue samples.

Chemical Residue Analyses

Several factors determine achievable detection limits for a specific contaminant, regardless of analytical procedure. These factors include:

- Size of the tissue sample available for analysis. In general, the more tissue available for analysis, the better the detection levels that can be achieved; a minimum of 30 g (wet weight) is usually considered adequate (USEPA, 1987c);
- Presence of interfering substances;
- Range of pollutants to be analyzed—an optimal method may be developed without regard to potential effects on other parameters;
- Level of confirmation—qualitative vs. quantitative analyses; and

- Level of pollutant found in the field and in analytical blanks.

Selection of appropriate methods ought to be based on a trade-off between full-scan analyses, which are economical but cannot provide optimal sensitivity for some compounds, and alternative methods that are more sensitive for specific compounds but can result in higher analytical costs. A list of analytical techniques is presented in Table 4-27.

The Computerized Risk and Bioaccumulation System (CRABS, Version 1.0) is an expert system designed to predict tissue residues of 15 neutral organic pollutants in sediment-dwelling organisms and the human cancer risk from consumption of contaminated shellfish (USEPA, 1990c). Thermodynamic partitioning, first-order kinetics, or toxicokinetic models are used to predict bioaccumulation from bedded sediment. Steady-state tissue residues are predicted from any of the models, whereas the two kinetic models are used to predict either non-steady-state uptake or elimination. The lifetime human cancer risk is then predicted for the consumption of clams and other nonmobile sediment-dwelling organisms containing the predicted or measured tissue residue. The cancer risk is predicted for a single pollutant from a single-species diet. Shellfish consumption rates can be estimated if site-specific rates are available.

Table 4-27. List of Analytical Techniques (USEPA, 1986b)

METALS/METALLOIDS	
• Atomic Absorption Spectrophotometry (AA)	USEPA 7000 series methods
- flame	
- graphite furnace (GFAA)	
- cold vapor	USEPA method 7470
- gaseous hydride (HYDAA)	USEPA methods 7060 and 7740
• Inductively Coupled Plasma Emission Spectrometry (ICP)	USEPA method 6010
ORGANICS	
• Gas Chromatography (GC)	
- with electron capture detection (GC/ECD)	USEPA method 8080
- with mass spectrometry (GC/MS)	USEPA methods 8240 and 8270

SOURCE: USEPA, 1986b.

This program is now available for distribution. All inquiries should be directed to the Narragansett, Rhode Island, Environmental Research Laboratory (Hatfield Marine Science Center, Newport, OR 97365, 503/867-4042).

Tissue residue data necessary for the evaluation of dredged materials and for ecological and human risk assessments are most directly derived using bedded sediments (i.e., deposited rather than suspended sediments) in bioaccumulation tests (USEPA, 1989d). Sediments are the largest receiving grounds for toxics known to bind with particles, such as organics with high octanol-water partitioning coefficients (e.g., PCBs and DDT) and many heavy metals. Previous techniques have varied to meet specific requirements for the task at hand, and comparability of results is questionable. USEPA (1989d) standardizes an approach for conducting sediment bioaccumulation tests using sediment-ingesting organisms exposed to bedded marine sediments. Guidance is presented for "routine" testing in the laboratory and has not been tailored for specific regulations or geographic locations. Data can be generated by the 28-day test that is applicable for quantitative ecological and human health risk assessments. It should be noted that this is a "living" document subject to revisions warranted by experience.

Metals and Metalloids

Nitric acid/perchloric acid digestions should be considered for analysis of tissue samples. Perchloric acid is especially useful for the dissolution of fat.

Trace element analyses by inductively coupled plasma emission spectrometry (ICP) allow for several elements to be measured simultaneously. However, the detection limits of ICP are generally not as sensitive as those achieved by graphite furnace atomic absorption spectrophotometry (GFAA).

The combination of atomic absorption spectrophotometry techniques (AA) and ICP is the recommended analytical method for detection of metals and metalloids since no technique is best for all elements. Cold vapor AA analysis is the only recommended technique for mercury.

GFAA is more sensitive than flame AA, but is more subject to matrix and spectral influences. GFAA requires particular caution with regard to laboratory contamination. In either case, the concentration of each element is determined by a separate analysis, making the analysis of a large number of contaminant metals both labor-intensive and relatively expensive compared to ICP.

Semivolatile Organic Compounds

Analysis of semivolatile organic compounds involves a solvent extraction of the sample, cleanup of the characteristically complex extract, and gas chromatography (GC) analysis and quantification. There are two gas chromatography/mass spectrometry

(GC/MS) options for detecting extractable organic compounds: internal standard technique and isotope dilution. Isotope dilution is recommended because reliable recovery corrections can be made for each analyte with a labeled analog or a chemically similar analog (Tetra Tech, 1986).

The identification of pesticides and PCB can be made by gas chromatography/electron capture detection (GC/ECD) analysis. GC/ECD provides greater sensitivity relative to GC/MS; however, GC/ECD does not provide positive compound identification. Confirmation of pesticides and PCBs on an alternative GC/ECD column or preferably by GC/MS, when sufficient concentrations occur, is recommended for reliable results (Tetra Tech, 1985). All other organic compound groups are recommended for analysis by GC/MS (Tetra Tech, 1985).

Volatile Organic Compounds

The purge-and-trap GC/MS technique is employed for detecting volatile organic compounds in water. A successful variation for detection of volatile organic residues in tissues involves a device that vaporizes volatile organic compounds from the tissue sample under vacuum and then condenses the volatiles in a super-cooled trap (Hiatt, 1981). The trap is then transferred to a purge-and-trap device, where it is treated as a water sample. The isotope dilution option is recommended because it provides reliable recovery data for each analyte (Tetra Tech, 1986).

4.10.4 QA/QC Considerations

Analysis of blanks should be conducted to demonstrate freedom from contamination. At least one method blank must be included with each batch of samples and must constitute at least 5 percent of all samples analyzed.

Spike recovery analyses are required to assess method performance on the sample matrix. This method serves as an indication of analytical accuracy, but not necessarily of extraction efficiency.

Replicates must be analyzed to monitor the precision of laboratory analyses. A minimum of 5 percent of the analyses should be laboratory replicates. Triplicates should be performed with each sample batch over 40 samples.

Laboratory performance and calibration should be verified at the beginning and end of each 12-hr shift during which analyses are performed.

Reports delineating the essential elements of the bioaccumulation component of the program should be included with the quantitative QA/QC analyses. It is recommended that these reports be recorded and stored in a data base for future reference.

4.10.5 Statistical Design Considerations

Composite Sampling

Composite tissue sampling consists of mixing tissue samples from two or more individual organisms, typically of a single species, collected at a particular location and time period. The analysis of a composite sample provides an estimate of an average tissue concentration for the individual organisms composing the composite sample. Advantages of the composite sampling strategy are :

- It provides a cost-effective strategy when individual chemical analyses are expensive.
- It provides a means to analyze bioaccumulation when the tissue mass of an individual is insufficient for the analytical protocol.
- It results in a more efficient estimate of the mean at specified sampling locations.

Because of the reduced sample variance, composite sampling results in a considerable increase in statistical power. If the primary objective of a monitoring program is to determine differences in contaminant tissue among sampling locations, composite sampling is an appropriate strategy.

Composite sampling is not recommended if the objective of the monitoring program is to determine compliance with specified tissue contaminant concentration limits since this sampling method does not detect the true range of tissue contaminant concentrations in the population. Special considerations related to composite sampling include :

- The range and the variance of the population of individual samples cannot be directly estimated.
- If species are mixed, tissue composites are likely to be composed of different proportions of species and numbers of individuals, confounding whether patterns of tissue residue concentrations are due to differences in locations or to interspecific differences in bioaccumulation.

Space-bulking consists of sampling of individual organisms from several locations and combining tissue samples into one or more composite samples. Time-bulking involves taking multiple samples over time from a single location and compositing these samples. The use of space- and/or time-bulking strategies should be carefully considered since significant information concerning spatial and temporal heterogeneity may be lost. The adoption of composite strategies will depend on the objective of individual monitoring programs.

Statistical Power

Tetra Tech (1987) demonstrated that the statistical power increases with the increase of the number of individuals in each replicate composite sample. However, a diminished return of statistical power exists with the addition of successive individuals to each composite. For composites of greater than 10 individuals, the increase of power is negligible given typical levels of data variability.

For moderate levels of variability in chemical residue data, 6 to 10 individuals composing each of 5 replicate composites should be adequate to detect a treatment difference equal to 100 percent of the overall mean among treatments (Tetra Tech, 1987). For analyses of individuals, a minimum of five organisms at each site is recommended.

To improve the power of a statistical test, while keeping the significance level constant, the sample size should be increased. Because of constraints in cost and time, however, this option may not be available. Power analyses have shown that for a fixed level of sampling effort, a monitoring program's power is generally increased by collecting more replicates at fewer locations. The number and distribution of sampling locations required to evaluate the effect of a discharge will depend on the volume and transport of the effluent.

4.10.6 Use of Data

Results of the bioaccumulation analyses can be used to :

- Establish spatial and temporal trends in the bioaccumulation of toxicants of selected marine fish and macroinvertebrates;
- Identify existing and potential problem areas for fish and macroinvertebrate contamination; and
- Supply data that can be used to calculate the human health risk of consuming marine fish and shellfish.

4.10.7 Summary

Rationale

- Monitoring the accumulation of chemical residues in tissues of marine organisms will provide information essential in relating the presence of selected contaminants in marine waters and sediment to their transfer and accumulation in marine organisms.

Monitoring Design Considerations

- Target species should possess the following characteristics :
  - high bioaccumulation potential for selected contaminants of concern;
  - weak or absent metabolic regulation of selected contaminants;
  - abundant enough, temporally and spatially, to allow for adequate sampling;
  - large enough to provide adequate amounts of tissue for analysis;
  - sessile or sedentary in nature to ensure bioaccumulation is representative of the study area; and
  - easily collected.
- Caged indicator species
  - Method allows for control of initial temporal and spatial variation of individuals and/or biomass.
  - Method allows for the use of specific age, size, and/or genetic stocks.
- Indigenous species
  - Results obtained will relate directly to those species that may be impacted.
- Target tissues
  - Fish liver or macroinvertebrate hepatopancreas analyses may be used to estimate the range of contaminants being assimilated.
  - Muscle tissue analyses are appropriate for human exposure assessments and quantitative health risk determinations.
  - Whole-body analyses should to be conducted when predators consume the whole body of the target organism.
- TOC/Lipid Normalization
  - Allow comparisons of chemical residue concentrations in tissues between locations, individuals, and tissue type.
  - Limitations in use due to differences in lipid analysis techniques.
- Time of Sampling
  - Timing of sampling should be based on biological cycles that influence an organism's susceptibility to bioaccumulate.
  - It is recommended that target species be sampled when tissue contamination concentrations are expected to be at their highest level.

Analytical Methods Considerations

- Metals/Metalloids
  - A combination of AA and ICP is recommended for the detection of metals and metalloids.
  - Cold vapor AA is the recommended protocol for mercury detection.
- Organics
  - GC/MS in conjunction with isotope dilution is recommended for the detection of semivolatile organic compounds.
  - Super-cooled trap, in conjunction with a purge-and-trap device, is recommended for the detection of volatile organics.

QA/QC Considerations

- Blank, spike recovery, and replicate analyses are recommended quality control checks.
- Reports delineating the essential elements of the bioaccumulation component of the program should be included with the quantitative QA/QC analyses.

Statistical Design Considerations

- Compositing tissue sampling consists of mixing tissue samples from two or more individual organisms collected at a particular location and time period.
- Space-bulking (combining composites from several locations) and time-bulking (combining several composites over time from one location) strategies should be used judiciously because information concerning spatial and temporal heterogeneity may be lost.
- Six individuals composing each of five replicate composites should be adequate to detect a treatment difference equal to 100 percent of the overall mean among treatments.

Use of Data

- Establish spatial and temporal trends in the bioaccumulation of toxicants of selected marine fish and macroinvertebrates.
- Identify existing and potential problem areas for fish and macroinvertebrate contamination.
- Supply data that can be used to calculate the human health risk of consuming marine fish and shellfish.

4.11 PATHOGENS

Monitoring for pathogenic microorganisms is currently conducted by State environmental and human health agencies in shellfish harvesting areas and bathing beaches. National Pollutant Discharge Elimination System (NPDES) pathogen monitoring programs may be under way at selected locations both to assess the condition of water in the vicinity of discharges and surrounding areas and to assess relative pathogen contributions from these permitted effluent discharges.

The National Shellfish Sanitation Program (NSSP) has been established as a joint effort involving the Food and Drug Administration (FDA), State agencies, and the shellfish industry to set forth guidelines for the management of State shellfish programs. As part of the NSSP, the Food and Drug Administration (FDA) provides technical assistance to States for studying specific pollution problems, provides data to establish closure levels for shellfish harvesting, conducts applied research on various contaminants to assist in developing standards and criteria, and evaluates the effectiveness of State shellfish sanitary control programs. In addition, since 1966, data have been compiled periodically by FDA and the National Oceanic and Atmospheric Administration (NOAA) on the classification by States of coastal and estuarine waters with regard to suitability for shellfishing activities. In addition to classifying their waters as to their suitability as shellfish harvesting areas, States also issue beach closures. These closures are typically based on water quality criteria developed by the Federal government.

4.11.1 Rationale

Monitoring of pathogens in the marine environment can be used in the CWA section 403 point source discharge program to address the following ocean discharge guideline:

- Potential direct or indirect impacts on human health.

Human pathogens found in the marine environment include viruses, bacteria, protozoans, helminth and parasites. In the United States, viruses and bacteria are the most important pathogens, in terms of both the number of organisms released to the environment and the severity of the diseases they cause (OTA, 1987).

Humans can be exposed to pathogens by direct contact with contaminated waters (e.g., swimming, surfing, diving) or indirectly through ingestion of contaminated food (e.g., molluscan shellfish). The preponderance of evidence indicates that the etiologic agents for waterborne outbreaks of acute gastroenteritis (AGI) are the Norwalk-like viruses (Kaplan et al. 1982). Hepatitis A has been linked to the consumption of raw or partially cooked molluscan shellfish (Feingold, 1973; CDC, 1979; Ohara et al., 1983). Bacteria responsible for typhoid and cholera are known to be water- and seafood-borne. These pathogens can enter the marine environment through the discharge of raw sewage, wastewater effluent from sewage treatment plants, failing septic tanks, and the dumping of sewage sludge (OTA, 1987). Monitoring of human pathogens provides information

essential in relating the presence of pathogens in marine waters and shellfish to outbreaks of disease. Monitoring data may be used to identify potential sources of pathogens. The assessment of pathogen contamination should be a component of a monitoring program where pathogens may present a risk to human health and the economic vitality of the marine environment.

4.11.2 Monitoring Design Considerations

Water Column Sampling

Bacteria are not uniformly distributed throughout the water column (Gameson, 1983); bacterial abundances, several orders of magnitude greater than underlying waters, can be found in a thin microlayer on the surface of the water (Hardy, 1982). If feasible, it is recommended that samples of this microlayer and separate samples of the underlying waters be collected. However, standardized methods for sampling this microlayer have not been established. If it is not feasible to sample both the microlayer and underlying waters, the "scoop" method should be used to ensure that the surface microlayer is sampled (USEPA, 1978).

Water samples for bacterial analyses are frequently collected using sterilized plastic bags (e.g., Whirl-Pak) or screw-cap, wide-mouthed bottles. Several depths may be sampled during one cast, and/or replicate samples may be collected at a particular depth by using Kemmerer or Niskin samplers (USEPA, 1978). Any device that collects water samples in unsterilized tubes should not be used for collecting bacteriological samples without first obtaining data that support its use. Pumps may be used to sample large volumes of the water column (USEPA, 1978).

Sentinel Organisms

Analyses of sentinel shellfish tissue (e.g., mussels and oysters) offer several advantages:

- They concentrate pathogens and may be useful viral analyses since viruses are often present in low numbers.
- They provide a means of temporally integrating water quality conditions.
- They may be direct measures of human exposure to pathogens (i.e., consumption of contaminated food).
- They may be deployed and maintained in a number of diverse locales.

However, the disadvantages of sentinel organisms include:

- Transplanting organisms may be costly;

- There is a possibility of losing the cage/buoy system; and
- Species may not be tolerant of all test site conditions (e.g., low salinity, high turbidity).

It is essential that monitoring design elements be standardized to allow for comparisons between marine studies. The selection of a standard sentinel species appropriate to the locality is recommended; interspecies differences would not allow comparisons of body burdens.

4.11.3 Analytical Methods Considerations

Many pathogens can be present in wastes, contaminated media, and infected organisms. Human pathogens are of concern in marine waters because they are:

- Associated with major debilitating diseases (e.g., hepatitis, cholera);
- Infectious at low doses;
- Resistant to environmental stress; and
- Not readily enumerated due to low numbers.

Table 4-28 provides examples of pathogenic organisms known to cause adverse human health effects. The alternative to directly identifying pathogens of concern has been the enumeration of bacteria, which are indicators of human waste contamination or indicators of human illness.

Indicators of Human Health Risks

Because of the inability to enumerate pathogens of concern, indicators of human pathogen densities have been used to assess human health risks. Indicators useful in predicting infectious disease rates should have the following characteristics:

- High abundances should be consistently found in human fecal wastes.
- No significant extra-human fecal sources should be present.
- The indicators must provide temporally and spatially reliable and accurate appraisals of the pathogen of concern.

Currently, there is no consensus on which indicator organism, if any, is specific for human feces.

Table 4-28. Microorganisms Responsible for Causing Adverse Human Health Effects[a]

Disease	Pathogenic Organism	Seafood Source
Hepatitis	Hepatitis A virus	Raw oysters Steamed clams Steamed and raw clams Raw clams Raw oysters Oysters Cockles Raw molluscan shellfish
	Non-A and non-B hepatitis virus	Raw molluscan shellfish
Gastroenteritis	*Aeromonas hydrophilia* and *Plesiomonas shigelloides*	Shellfish
	Vibrio mimicus	Raw oysters
	Vibrio parahaemolyticus	Clams and snails Raw oysters Crab Shrimp Lobster
	Vibrio vulnificus	Raw oysters
	Vibrio cholera, O group	Raw oysters Boiled shrimp, boiled crab
	Vibrio cholera, Non-O group 1	Raw oysters
	Norwalk virus	Raw oysters Raw clams
	Small round structured virus	Raw oysters
	Campylobacter jejuni	Raw clams

[a] Includes naturally occurring microorganisms as well as microorganisms associated with pollution.
SOURCE: NOAA, 1988

Coliform Bacteria

For decades, the concentration of coliform bacteria, either total or fecal coliform, has been considered a reliable indicator of the presence and density of pathogens. The use of total coliform bacterial criteria to protect human health from disease associated with contaminated water is widespread. Furthermore, total coliform concentrations have the advantage of providing a basis for comparison with historical data (USEPA, 1988f).

However, these indicators and their corresponding water quality standards have not been related to incidences of disease through epidemiological studies. Controversy exists on the efficacy of coliform bacteria to predict the presence and inactivation of other types of pathogens (Pederson, 1980). In fact, recent studies indicate that fecal coliform bacteria may not be a reliable indicator for predicting the risks associated with direct exposures to pathogens in the marine environment (Cabelli et al., 1979, 1982). Fecal coliforms are not pathogenic and are less resistant to environmental stress compared to many pathogens (Borrego et al., 1983). Furthermore, fecal coliform bacteria are not specific to mammalian fecal pollution. The lack of this specificity prompted the development of methods for enumerating fecal coliform bacteria specific to mammalian fecal pollution (e.g., *Escherichia coli*). However, with respect to recreational water quality criteria, EPA has not recommended the use of *E. coli* for marine waters.

Enterococci

Enterococci are streptococcus bacteria indigenous to the intestines of warm-blooded animals. Cabelli et al. (1983) found that the densities of enterococci were highly correlated with the incidence of gastrointestinal (GI) symptoms among swimmers; reported swimming-associated GI symptoms were poorly correlated with fecal coliform densities. Enterococci also have the following advantageous characteristics:

- They are tolerant to high salinity and are of particular value in the analysis of marine waters.
- Taxonomic identifications are relatively simple and can reveal the origin of mammalian pollution (e.g., humans, livestock).
- Genetic fingerprinting techniques that can link these bacteria in the environment to specific sources of contamination have been developed.

State-of-the-art genetic fingerprinting techniques are very costly and require further study in order to assess their reliability. EPA has adopted enterococci as an indicator of microbiological water quality for recreational marine waters.

Viruses

Viruses are being recognized as major etiologic agents for many outbreaks of human illness as a result of swimming in contaminated water and consuming contaminated seafood. Viruses are excreted only by infected individuals; they are not normal flora in the intestinal tract. Traditional indicators of microbial contaminants appear to be inadequate for predicting human health risks associated with both consuming molluscan shellfish and swimming in waters that contain sewage-associated viruses. However, examination of water for enteric viruses is not recommended at this time, except in special circumstances, because of methodology limitations. Even state-of-the-art methods for concentrating viruses from water are still being researched and continue to be modified and improved. None of the available virus detection methods have been tested adequately with representatives from all of the virus groups of public health importance. In addition, some of these methods require expensive equipment and materials for sample processing and all virus assay and identification procedures require expensive cell culture and related virology laboratory facilities.

Laboratory Techniques

It should also be noted that no single procedure is adequate to isolate all microorganisms from water, and the presence of one microorganism does not signify the presence or absence of any other (Table 4-29). A more detailed description of analytical methods for a number of pathogens is presented in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1989).

Fecal Bacteria

Two standard methods are presented here for the detection of fecal bacteria: the membrane filter procedure and the multiple-tube fermentation procedure.

The membrane filter (MF) technique involves sample filtration followed by direct plating for detection and enumeration of coliform bacterial densities. The MF technique can be used to test relatively large volumes of samples and yields numerical results more rapidly than does the multiple-tube procedure. The statistical reliability of the MF technique is greater than that of the Most Probable Number (MPN) procedures (APHA, 1989). However, the MF technique has limitations, particularly in testing waters with high turbidity and noncoliform (background) bacteria. The MF technique can be used to measure bacterial densities of *Escherichia coli* (*E. coli*) and enterococci in ambient waters (USEPA, 1985e) since EPA has approved this technique for use in seawater.

The multiple-tube fermentation technique involves a series of fermentation tubes containing growth media that are inoculated with the appropriate decimal dilutions of water (multiples and submultiples of 10 mL), based on the probable coliform density. Following inoculation at 35 ± 0.5°C for 24 ± 2 hr, the tubes are examined for gas or acidic growth (distinctive yellow color). If no gas or acidic growth has formed, the

Table 4-29. Laboratory Procedures for Bacterial Indicators

Test Organisms	Laboratory Procedures		
	Water	Sediment	Tissue
Fecal coliform bacteria	MPN tubes using A-I broth (APHA, 1989) (fecal coliform bacteria/100 mL)	MPN tubes using A-I broth (APHA, 1989) (fecal coliform bacteria/100 mL)	MPN tubes using EC broth (APHA,1989) (fecal coliform bacteria/100 mL)
Fecal coliform bacteria/*E.coli*	mTEC (DuFour et al., 1981) (*E. coli*/100 mL)	-	-
Enterococci	mE (Levin et al., 1975)[a] (enterococci/100 mL)	-	-
C. perfringens[b]	MPN tubes using iron milk (St. John et al., 1982) (*C. perfringens*/ 100 mL)	MPN tubes using iron milk (St. John et al., 1982) (*C. perfringens*/ 100 mL)	MPN tubes using iron milk (St. John et al., 1982) (*C. perfringens*/ 100 mL)

[a]This method is a tedious process. EPA Region 2 and the State of New Jersey have developed a modified mE isolation technique.

[b]Two laboratory techniques are available for *C. perfringens*: mCP by membrane filtration for water (Bisson and Cabelli, 1979) and sediment (Emerson and Cabelli, 1982), and iron milk tube using MPN techniques (St. John et al., 1982). The latter method is recommended (pending comparative data) because the procedure is simpler and less costly.

samples are reincubated and examined again at the end of 48 ± 3 hr. Production of gas or acidic growth on tubes after this time period constitutes a positive presumptive reaction. These samples are then submitted to the confirmed phase of testing, in which the culture is transferred to a fermentation tube containing brilliant green lactose bile broth. The tubes are then inoculated for 48 ± 3 hr at 35 ± 0.5°C. Formation of gas in any amount of time constitutes a positive confirmed phase. The Most Probable Number (MPN) index is then used to estimate bacterial density. This technique is commonly used in assessing bacterial levels in shellfish.

The MPN index is an index of the number of coliform bacteria that, most probably, would produce the results observed in the laboratory examination (APHA, 1989). Values for the MPN index can be obtained from standard tables based on the results of the multiple-tube fermentation technique or from Thomas's formula (APHA, 1989).

Viruses

Detecting viruses in marine waters requires collecting a representative water sample, concentrating viruses in the sample, and identifying and estimating abundances of these concentrated viruses. Difficulties in detecting virus abundances include:

- Viruses are very small (20-100 nm).
- Virus concentrations in waters are spatially and temporally variable, and typically low.
- Dissolved and suspended material in the sample interferes with accurate virus detection.

Because virus concentrations may be very low, significant volumes of water may be required (e.g., on the order of tens to thousands of liters). Dose-response curves are lacking for most pathogens; however, as few as 10 to 100 bacteria are capable of inducing disease (OTA, 1987).

Three different techniques for concentrating and enumerating viruses are presented in *Standard Methods* (APHA, 1989):

- Adsorption to and elution from microporous filters;
- Aluminum hydroxide adsorption and precipitation; and
- Polyethylene glycol hydroextraction-dialysis.

The adsorption and elution technique pressure-filters viruses on microporous filters and then elutes them from the filter in a small liquid volume. Generally, two types of filters are available: electronegative and electropositive filters. Currently, insufficient documentation on the efficacy of electropositive filters exists. Limitations of this technique include:

- The adsorbent filter may be clogged by suspended material.
- Dissolved colloid material may interfere by competing with viruses for adsorption sites on the filter.
- Viruses adsorbed to suspended material may be removed during suspended material clean-up procedures.

Methods for recovering solids-associated viruses are found in APHA (1989). In spite of these limitations, the adsorption and elution technique remains the most promising method for detecting viruses.

Aluminum hydroxide adsorption and precipitation and polyethylene glycol hydroextraction-dialysis are used to reconcentrate viruses in proteinaceous and organic buffer eluates. These methods may be used to concentrate viruses in waters having high virus densities (e.g., wastewaters). They may also be used as a second-step concentration procedure following processing of large fluid volumes through microporous filters. However, these two techniques are impractical for primary processing of large fluid volumes (APHA, 1989).

New Techniques

Traditional techniques lack the ability to detect apparently viable, but nonculturable, microorganisms. Recently developed monoclonal antibody and gene probe techniques permit the detection and enumeration of both culturable and nonculturable microorganisms. Since these methods have the ability to detect nonculturable organisms, they may serve as more precise techniques for monitoring microbial water quality. Furthermore, these new techniques will allow direct monitoring of pathogens of concern. The goal of monitoring specific pathogens, such as *Salmonella*, *Shigella*, *Giardia*, or *Legionella*, may soon be realized.

Sample Handling

Preservation and storage of water samples can be significant sources of error. Sample bottles must be resistant to sterilization procedures. Samples should be refrigerated (1-4°C) during transport to a laboratory and analyzed within 6 hours of collection (USEPA, 1978).

4.11.4 QA/QC Considerations

It is recommended that sterile distilled water be transported to the field, transferred to a sample bottle, and processed routinely as a field blank to ensure that samples were not contaminated during collection and transport. It is also recommended that 10 percent of the samples be analyzed in duplicate. Furthermore, 10 percent of the samples should be split and analyzed by two or more laboratories.

Intralaboratory and interlaboratory quality control practices should be documented, and QC reports should be available for inspection. Further recommended quality assurance guidelines for a microbiology laboratory are available (Inhorn, 1977; Bordner et al., 1978) and are also discussed in APHA (1989).

4.11.5 Statistical Design Considerations

Bacterial counts often are characterized as having a skewed distribution because of many low values and a few high ones (APHA, 1989). Application of parametric statistical techniques requires the assumption of symmetrical distributions such as the normal curve. An approximate normal distribution can be obtained from positively skewed data by converting numbers to their logarithms (APHA, 1989). Accordingly, the preferred statistic for measuring central tendency of microbiological data is the geometric mean.

Consideration of statistical strategies will mitigate the high costs of collecting and processing samples. Power-cost analyses are necessary in selecting appropriate sample/replicate number, sample location, and sampling frequency (Ferraro et al., 1989).

4.11.6 Use of Data

The assessment of pathogen contamination should be a component of a monitoring program where pathogens may present a risk to human health and the economic vitality of an estuary. Monitoring of human pathogens provides information essential in relating the temporal and spatial distribution of infectious agents in marine waters and shellfish to the epidemiology of pathogens of concern (e.g., affected human populations, locations, and timing of the outbreak).

Furthermore, monitoring data can be used to identify discharges that may be significant sources of pathogens and to ensure that water quality standards are maintained. Monitoring data can also be used to verify fate and transport, human health risk assessment, and epidemiological modeling predictions.

4.11.7 Summary and Recommendations

Rationale

- The objective is to detect and describe spatial and temporal changes in abundances of indicators of human health pathogens.
- Monitoring indicators of human pathogens provides information essential in relating the presence of infectious agents in marine waters and shellfish to the incidence of disease outbreaks and potential sources of these agents.

Monitoring Design Considerations

- If feasible, samples of the surface microlayer should be collected separately from samples of underlying waters. If not feasible, samples containing both underlying and microlayer waters should be collected. It is highly recommended that consistent types of sampling protocols be implemented to allow for comparisons between studies.
- Analysis of tissues of sentinel organisms (e.g., mussels and oysters) confers the following advantages:
  - such organisms concentrate pathogens;
  - such organisms provide a means of temporally integrating water quality conditions; and
  - such organisms may be deployed and maintained in a number of locales.

Analytical Methods Considerations

- Indicators of human pathogens should have the following characteristics:
  - should be consistently found in high abundance in human fecal wastes;
  - should not have significant extra-human fecal sources; and
  - must provide temporally and spatially reliable and accurate appraisals of the pathogen of concern.
- Fecal coliform bacteria densities.
  - May not be a reliable indicator for predicting the risk associated with direct exposures to pathogens.
  - This measure provides a means to compare current data to historical data.
  - Laboratory analyses include membrane filter and multiple-tube fermentation techniques.
- Enterococci densities
  - Densities are highly correlated with the incidence of gastrointestinal symptoms.
  - Taxonomic identifications are relatively simple and can reveal the kinds of mammalian pollution.
  - State-of-the-art genetic fingerprinting techniques can link bacteria in the marine environment to specific sources; however, these techniques are very costly and require further study to assess their reliability.
  - Laboratory analyses include membrane filter technique.
- Virus densities
  - Viruses are recognized as major etiologic agents for many outbreaks of human illness.

  - Examination of water samples for enteric viruses is not recommended because of methodology limitations.
  - Laboratory analyses include absorption to an elution from microporous filters, aluminum hydroxide absorption-precipitation, and polyethylene glycol hydroextraction-dialysis.

- Monoclonal antibodies and gene probes show promise in detecting and enumerating both culturable and nonculturable microorganisms.

QA/QC Considerations

- Sterile distilled water should be transported and transferred to sample bottles as field blanks to assess contamination during collection and transport.
- Ten percent of the samples should be analyzed in duplicate.
- Ten percent of the samples should be split and analyzed at two or more laboratories.

Statistical Design Considerations

- The preferred statistic for measuring central tendency of microbiological data is the geometric mean.

Use of Data

- Provide essential information to assess threats to human health;
- Establish temporal and spatial trends in pathogen densities and identify potential relationships between the presence of the indicator and incidence of human illness; and
- Provide information that can be used to verify fate and transport, human health risk, and epidemiology models.

4.12 EFFLUENT CHARACTERIZATION

"End-of-pipe" effluent characterizations based on laboratory studies can be used to predict biological impacts of an effluent prior to a discharge. The *Technical Support Document for Water Quality-based Toxics Control* (USEPA, 1991c) presents (among other things) an integrated approach for ensuring protection of aquatic life and human health from impacts caused by the release of toxics to surface waters. For the protection of aquatic life, the integrated strategy involves the use of three control approaches: the chemical-specific control approach, the whole-effluent toxicity control approach, and the biological criteria/bioassessment and biosurvey approach. For the protection of human health, technical constraints do not yet allow for full reliance on an integrated strategy, and thus primarily chemical-specific assessments and control techniques should be employed. Table 4-30 contains a list of terms used in effluent characterization.

Pollutant-specific criteria developed pursuant to CWA section 304(a)(1) present scientific data and guidance on the environmental effects of pollutants that reflect the latest EPA recommendations on acceptable limits for aquatic life and human health protection. These limitations are generally developed from laboratory-derived, biologically-based numeric water quality criteria adopted within a State's water quality standards. Water quality criteria are adopted by a State for the protection of designated uses of the receiving water. Criteria can be specific numeric limits or, in the absence of specific numeric criteria for a chemical, biological, or physical parameter, can be based on narrative criteria. EPA publishes chemical-specific water quality criteria that may provide the basis on which States adopt their criteria (USEPA, 1986-1988). EPA has published 25 chemical-specific saltwater aquatic life criteria (acute and chronic).

When an effluent's constituents are not completely known or when a complex mixture of potentially additive, antagonistic, or synergistic toxic pollutants is discharged, a whole-effluent toxicity limitation can be implemented. The whole-effluent toxicity limitation can also be appropriate when more than one discharger is located in a specific area and the potential exists for effluent mixing and additive toxic effects, and when a pollutant-specific evaluation is impractical because of a lack of information about the toxic effects of a pollutant. Toxicity evaluations such as these can be used as part of 403 monitoring.

4.12.1 Rationale

Effluent characterization can be used in the CWA section 403 point source discharge program to address the following evaluation criteria:

Table 4-30. List of Terms Used with Effluent Characterization

Acute	A stimulus severe enough to rapidly induce an effect; in aquatic toxicity tests, an effect observed in 96 hours or less typically is considered acute. When referring to aquatic toxicology or human health, an acute effect is not always measured in terms of lethality.
Acute-to-chronic ratio (ACR)	The ratio of the acute toxicity of an effluent or a toxicant to its chronic toxicity. ACR is used as a factor for estimating chronic toxicity on the basis of acute toxicity data, or the reverse.
Chronic	A stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. Chronic should be considered a relative term depending on the life span of an organism. The measurement of a chonic effect can be reduced growth, reduced reproduction, etc., in addition to lethality.
Composite sample	A single effluent sample collected over a 24-hour period, on which only one toxicity test is performed.
Effective concentration	A point estimate of the toxicant concentration that would cause an observable adverse effect (such as death, immobilization, or serious incapacitation) in a given percentage of the test organisms.
Grab samples	Samples collected over a very short period of time and on a relatively infrequent basis. A separate toxicity test must be performed on each grab sample.
Lowest-Observed-Adverse-Effect Level (LOAEL)	The lowest concentration of an effluent or toxicant that results in statistically significant adverse health effects as observed in chronic or subchronic human epidemiology studies or animal exposure.
No-Observed-Adverse-Effect Level (NOAEL)	A tested dose of an effluent or a toxicant below which no adverse biological effects are observed, as identified from chronic or subchronic human epidemiology studies or animal exposure studies.
No-Observed-Effect Concentration (NOEC)	The highest tested concentration of an effluent or a toxicant at which no adverse effects are observed on the aquatic test organisms at a specific time of observation. Determined using hypothesis testing.
Toxicity characterization	A determination of the specific chemicals responsible for effluent toxicity.
Whole-effluent toxicity	The aggregate toxic effect of an effluent (usually measured directly with a toxicity test).

- The quantities, composition, and potential for bioaccumulation or persistence of the pollutants to be discharged;
- The potential impact on human health through direct and indirect pathways; and
- Marine water quality criteria developed pursuant to section 304(a)(1).

Assessment of these evaluation criteria can be made through whole-effluent toxicity testing (acute and chronic) and analysis of the chemical composition of the effluent. This assessment would demonstrate potential impacts from the effluent in question.

4.12.2 Monitoring Design Considerations

Determination of whether an effluent sample is typical of the wastewater may require the collection of a large number of samples. Further, what constitutes a "representative" sample is a function of the parameter of concern. Guidelines for determining the number and frequency of samples required to represent effluent quality are contained in the *Handbook for Sampling and Sample Preservation of Water and Wastewater* (USEPA, 1982b).

Both quantitative (change in concentration) and qualitative (change in toxicants) variability commonly occur in effluents. Changes in effluent toxicity are the result of varying concentrations of individual toxicants, different toxicants, changing in-stream water quality characteristics (affecting compound toxicity), and analytical and toxicological error. Conventional parameters, BOD, TSS, and other pollutants limited in the facility's permit will provide an indication of the operational status of the treatment system on the day of sampling. This information may be used in a determination as to whether the system is operating properly, requires repair, or needs to be upgraded. For industrial discharges, information on production levels and types of operating processes may be helpful in determining the required magnitude of the monitoring program.

4.12.3 Analytical Methods Considerations

Sampling

The choice of grab or composite samples will depend on the specific discharge situation (e.g., plant retention time) and the objectives of the test. In toxicity characterization testing, samples that are very different from one another give results that are difficult to interpret. However, composite sampling has an averaging effect, which tends to dilute toxicity peaks and may thus provide misleading results when testing for acute toxicity. Composited samples, therefore, are more appropriate for chronic toxicity tests where peak toxicity of short duration is of less concern. If the toxicity of the effluent is variable, grab samples collected during peaks of effluent toxicity provide a measure of maximum effect. However, while grab sampling may provide information on maximum effluent

toxicity, it can be difficult to schedule sampling to coincide with peaks in toxicity. Aeration during collection and transfer of effluents should be minimized to reduce the loss of volatile chemicals.

Chemical-Specific Analysis

Pollutant-specific testing for comparison to water quality criteria should be done following standard methods. Choice of a particular method may vary depending on the precision and accuracy required and the resources available for the study. A review of water chemistry analytical methods is presented in the section in this report on water quality and in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1989). However, it should be noted that pollutant concentrations are considerably higher in effluent streams and the precision required for adequate measurement is lower.

Whole-Effluent Toxicity Analysis

Whole-effluent toxicity testing can be somewhat more complex than a pollutant-specific approach. Upon arrival of the sample in the laboratory, temperature, pH, hardness, and conductivity should be measured. Total residual chlorine, total ammonia, alkalinity, dissolved oxygen (DO), and organic carbon measurements may also be appropriate. These measurements can provide necessary information should the toxicity of the effluent change over time.

EPA recommends that for whole-effluent toxicity data generation the process should be divided into three basic steps: initial dilution determination, toxicity testing procedures, and triggers for permit limit development. The dilution determination is an estimate of the effluent dilution at the edge of the mixing zone and should take any applicable State mixing zone requirements into consideration.

Whole-effluent toxicity testing should entail both acute and chronic testing. An acute toxicity test is defined as a test of usually less than 96 hours in duration in which lethality is the measured endpoint. A chronic toxicity test is defined as a long-term test in which sublethal effects, such as fertilization, growth, and reproduction are usually measured, although in highly toxic effluents lethality may also result. Traditionally, chronic tests are full-life-cycle tests or at least 30-day tests. However, the duration of most of the EPA chronic toxicity tests has been shortened to 7 days by focusing on the most sensitive life-cycle stages. For this reason, the EPA chronic toxicity tests are called short-term chronic tests. Whole-effluent toxicity limits can be inserted into permits as a means of controlling pollutants when chemical-specific criteria have not been developed or synergistic effects are found to occur.

For whole-effluent toxicity testing, EPA recommends that three species (a vertebrate, an invertebrate, and a plant) be tested quarterly for a minimum of 1 year. This may be adjusted to require testing of only the most sensitive species. Conducting tests quarterly for 1 year is recommended to adequately assess the variability of toxicity observed in effluents. The use of three of the five commonly used marine organisms—inland silverside, sheephead minnow, mysid shrimp, *Champia*, and sea urchin—has generally been sufficient to measure the toxicity of any effluent for the purposes of projecting effluent toxicity impact and making regulatory decisions. Specific test methodologies can be found in a variety of EPA manuals, including *Methods for Aquatic Toxicity Identification Evaluations* - Phases I, II, and III (USEPA, 1988a, b, c), *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms* (USEPA, 1985c), *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms* (USEPA, 1988e), and *Biomonitoring for Control of Toxicity in Effluent Discharges to the Marine Environment* (USEPA, 1989b). Numerous other sources of toxicity testing methodologies exist (see reference list) and may be more appropriate for the effluent of interest.

Biological Criteria/Bioassessment

To fully protect aquatic habitats, water quality criteria should address biological integrity. Biocriteria should be used as a supplement to existing chemical-specific criteria and as criteria where such chemical-specific criteria have not been established. Biocriteria are numerical values or narrative expressions that describe the reference biological integrity of aquatic communities inhabiting unimpaired waters. The biological communities in these waters represent the best attainable conditions (USEPA, 1991c).

Resident biota integrate multiple impacts over time and can detect impairment from known and unknown causes. Biocriteria can be used to verify improvement in water quality in response to regulatory efforts and detect continuing degradation of waters. Numeric criteria can provide effective monitoring criteria for inclusion in permits (USEPA, 1991).

The assessment of biological integrity should include measures of the structure and function of an aquatic community of species within a specified habitat. Expert knowledge of the system is required for the selection of appropriate biological components and measurements indices. See Section 4.5 for further discussion of benthic community structure.

4.12.4 QA/QC Considerations

Basic sampling and laboratory quality assurance and quality control (QA/QC) procedures for pollutant-specific characterization are addressed in the section on water chemistry. QC problems specific to effluent characterization include variability in effluent concentrations and toxicity response of test organisms, and changes in effluent toxicity

over time. A quality assurance project plan (QAPjP) should be developed and adhered to. The QAPjP should include quality verification, which entails a demonstration that the proposed study plan was followed as detailed and that work carried out was properly documented. The QAPjP should increase communication between clients, program planners, field and laboratory personnel, and data analysts. The QAPjP must make clear the specific responsibilities of each individual. The QC procedures involve standardized guidelines, such as the number of samples to be taken and the mode of collection, standard operating procedures for analyses, and spiking protocols. QC protocols for standard toxicity test methods are described in USEPA (1985c).

4.12.5 Statistical Design Considerations

Statistical design of an effluent characterization project must account for variability in effluent and toxicity testing response. However, the design of any given experiment must be weighed against the importance of the data and decisions to be based on the data. The critical nature of certain data will demand stringent controls, while statistical rigor (and associated costs) can be lessened in other experiments having less impact (e.g., initial toxicity testing).

The choice of a statistical method to analyze toxicity test data and the interpretation of the results of the analysis of the data from toxicity tests can be problematic because of the inherent variability and sometimes unavoidable anomalies in biological data. The assistance of a trained statistician is recommended before selecting the method of analysis and interpretation of the results. The data should be plotted to help detect problems and unsuspected trends or patterns in the responses and to aid in interpretation of the results. The analysis of the data is dependent upon the number of replicates and the distribution and homogeneity of the data. A variety of statistical methods for the analysis of toxicity data (including Dunnett's procedure, Bonferroni's t-test, Steel's many-one rank test, the Wilcoson Rank Sum test, and the Probit analysis) are presented in USEPA (1988e).

4.12.6 Use of Data

Pollutant-specific effluent characterization can be used for comparison to State water quality standards. A chronic criterion is a level at which aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of the pollutant is not exceeded more than once every 3 years on average. An acute criterion is a limit at which the pollutant concentration should not be exceeded more than once every 3 years on average. Care should be taken when applying these criteria to assess potential impacts on locally important species that are very sensitive.

Whole-effluent toxicity testing data (both acute and chronic) may be used to screen an effluent for potential unreasonable degradation of the environment. Whole-effluent toxicity is a useful parameter for assessing and protecting against impacts on water

quality and designated uses caused by the aggregate toxic effect of the discharge. Thus, toxicity itself is used as the effluent parameter, and the toxicants creating the toxicity need not be specifically identified or controlled unless a Toxicity Reduction Evaluation (TRE) is required to ensure compliance with a toxicity limit in the permit.

If a threshold of toxicity is desired, then the results of whole-effluent toxicity testing may be used to derive the No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC). Whole-effluent toxicity testing can also produce a concentration-dependent result of some amount of adverse effect called the Effective Concentration (EC). For example, EC_{50} is the effluent concentration that would affect 50 percent of the organisms tested. Interpretation of EC values, therefore, requires the judgment of a toxicologist.

4.12.7 Summary and Recommendations

Rationale

- Effluent characterization may be used to assess potential impacts, both acute and chronic, of the effluent on the surrounding biological communities without interference from other sources.

Monitoring Design Considerations

- Effluent variability, including potential changes in contaminants and contaminant concentrations, should be considered when designing a monitoring plan.

Analytical Methods Considerations

- Grab or composite samples should be used depending on the objectives and type of test performed.
- Chemical-specific testing can be performed to characterize toxicity and ensure compliance with water-quality standards.
- Both acute and chronic tests can be used to assess whole-effluent toxicity.

QA/QC Considerations

- See section on water chemistry.
- QAPjP should be developed, adhered to, and documented.

Statistical Design Considerations

- Effluent variability and toxicity testing response must be accounted for in the monitoring plan.

Use of Data

- Chronic and acute toxicity levels can be set in permits.
- Toxicity Reduction Evaluations (TREs) can be required.
- Threshold and effective concentrations can be established to predict ecological risk.

4.13 MESOCOSMS AND MICROCOSMS

The use of mesocosms and microcosms for the assessment of ecological impacts from marine dischargers is considered to be premature. This monitoring approach is the focus of research and holds promise for future use. It has been used to some extent by EPA's Office of Pesticides to predict impacts from the use of pesticides.

Microcosms and mesocosms, on a small to medium size scale, respectively, attempt to simulate the complex nature of natural environments. An effort is made to determine the response of an ecosystem to perturbation through an accurate simulation of the physical, chemical, and biological characteristics of an ecosystem and the biological interactions of the organisms in the ecosystem. While there is no absolute separation between a mesocosm and a microcosm, in practice most mesocosms are outdoors where temperature and light intensity are ambient for the parent community. Most mesocosm researchers try to realistically scale for the factors they think are most important in controlling ecological relationships. Most microcosms are incubated in the laboratory and are subject to greater environmental control. Microcosms are usually smaller, and more replicates are used.

4.13.1 Rationale

Of the 10 guidelines used to determine unreasonable degradation or no irreparable harm, several can be addressed through the use of mesocosms and microcosms including:

- The potential transport of such pollutants by biological, physical or chemical processes and
- The composition and vulnerability of potentially exposed biological communities.

Mesocosms and microcosms can be used to gain a realistic characterization of ecosystem-level impacts of marine discharges subject to section 403. With mesocosms and microcosms, the responses of complete ecosystems to pollutants can be investigated. Other advantages include the possibility of experimentation, at near natural conditions, with addition of isotopic tracers to an ecosystem and the opportunity to test numerical transport models under near natural conditions.

If insufficient information exists to determine whether a discharge will result in unreasonable degradation of the marine environment, EPA must make a determination as to whether a discharge will cause irreparable harm during the period in which monitoring is undertaken. The use of microcosms allows for the incorporation of ecosystem recovery (a critical factor in making a determination of irreparable harm) as an endpoint for future ecological risk assessment (Perez et al., 1990).

4.13.2 Monitoring Design Considerations

A microcosm should be designed to represent a miniature ecosystem that responds quickly to perturbation. The emphasis is on ecosystem-level properties, although species-level attributes such as fecundity and mortality can also be monitored if desired. Most generic microcosms do not simulate a site-specific natural system, but function as very generalized simulations of a large class of ecosystems. However, the use of undisturbed, natural aquatic, and benthic communities (through the use of a sediment core) in a single system allows for the simulation of a natural system (USEPA, 1983b). The design of the microcosm allows the system to be defined in terms of its physical and temporal boundaries, its light and temperature regime, its water composition, the turbulence and turnover rate, the ratio of benthic surface area to seawater volume, the sediment characteristics, and the water flow rate over the sediment surface.

The results of microcosm or mesocosm experiments are measured by the ecological effects on the biota of the system. When selecting biota to include in the test, two factors should be considered, both of which involve selection of the benthic component of the system. If the natural system has more than one distinct benthic community, then those organisms which are directly linked to human consumption or those which are important to the economics and aesthetics of the area should be considered for experimental use. Moreover, if some of the benthic communities contain species known to be more sensitive to environmental contaminants than others, these communities should also be considered.

4.13.3 Analytical Methods Considerations

Microcosms for which ecosystem effects protocols have been developed are small, static, open ecosystems. Kenneth Perez and other researchers at the EPA Environmental Research Laboratory in Narragansett, Rhode Island, developed an experimental marine microcosm test protocol that employs a time frame of 30 days (USEPA, 1983b). The methodology was published in the *Federal Register* (volume 52, number 187, pages 36352-36360) for use in developing site-specific data on the chemical fate and ecological effects of chemical substances subject to regulation under the Toxic Substances Control Act (TSCA) and could be adapted to the 403 ocean discharge program. This protocol attempts to couple undisturbed pelagic and benthic communities within a single system, the physical and chemical conditions of which are equated to those in the natural system being simulated. The protocol and support document (USEPA, 1990e) describes the steps necessary to develop the experimental microcosm (tanks, paddles, benthic cylinders, pumps, and pump air supply) and support equipment (room, water bath, light and turbulence fixtures, test compartments, and an air evacuation system). A diver-collected sediment core is used for the benthic subsystem, and the test water is collected by a nondestructive method and exchanged at least three times a week, coinciding with biological and chemical sample collection. Water flow, turbulence, light intensity, temperature, and simulated tidal flow are all controlled to simulate the natural system for the 30-day time period.

Other researchers contend that to study ecosystem-level effects it is essential that the microcosms function (1) as homeostatic, self-sustaining ecosystems capable of existing through at least a year and (2) independent of outside subsidies except for light and replacement of evaporated water. Each microcosm is considered a unique ecosystem and intraexperiment replication among microcosms is enhanced by using defined chemical media and standard physical conditions (light, temperature, day length). Inoculation with well-developed interactive couplings of the organisms is essential to achieve these goals. Selection of the exact species to be used should be based on the specific objectives of the study.

During microcosm studies, effluent to be tested should be added on a gradient. A typical experimental design might consist of six replicates in each of four treatment groups: a control, a single addition of a low concentration, a single addition of a high concentration, and repeated additions of low concentrations. Example protocol steps and the variables monitored for this type of generic microcosm are described in Taub (1984).

The physical structure of a mesocosm system determines the ability of water, organisms, and pollutants to move from compartment to compartment within the system. Coastal ecosystems and the fate and effects of pollutants within these systems are strongly affected by vertical mixing and the interaction of planktonic and benthic processes. Donaghay (1984) classified mesocosm systems by their degree of planktonic/benthic coupling into three categories: single well-mixed benthic coupled, single stratified, and totally benthic decoupled. Single well-mixed benthic coupled systems are composed of a well-mixed water column in direct contact with sediment and benthos. This system has been used extensively at the Marine Ecosystem Research Laboratory (MERL) with very good results validating field data; defining loadings, fates, and effects; and manipulating natural systems to define underlying processes. The single stratified system design involves well-mixed top and bottom layers separated by an unmixed thermocline with only the bottom layer in contact with the sediment. The system is used to study systems where stratification is important. The single system without benthic coupling is intended to model fates and effects in stratified deep water systems without benthic coupling.

4.13.4 QA/QC Considerations

In some cases the accuracy of the results of mesocosms and microcosms may be limited by a number of factors. The size of the systems most often excludes macrofauna such as fish and large crustaceans. The size of a microcosm can also affect the mixing rates of surficial sediments. An increase in the size of a microcosm will result in an increase in exposure of sediment particles to the overlying water column. Results from smaller-sized systems have underestimated potential risks of complex effluent (Perez et al., in press; Pontasch et al., 1989); however, attempts are being

made to develop "scaling laws" to describe this relationship. These systems are also limited to photo-stable test substances because the light intensity may be higher than that which would occur naturally.

4.13.5 Statistical Design Considerations

The results of the microcosm and mesocosm experiments assist in determining the fates and ecological effects of contaminants within a marine system. The pelagic biota are characterized by the number and species composition of phytoplankton, zooplankton, and transient larval forms. The benthic community is characterized by the structural composition (see Section 4.5, Benthic Community Structure). The experimental marine test protocol (USEPA, 1990e) recommended test design and statistical analysis allow for the independent assessment of the solvent carrier (if used) and the effluent for all variables measured. Also, the differences in the biotic response between the control microcosms and the natural system will provide a measure of the validity of the test response. A multivariate analysis of variance, followed by univariate analysis, and regression techniques are recommended for the analysis of all data.

Statistical design considerations of mesocosms must consider that the scientific and financial resources for mesocosm studies are limited. Therefore, efforts must be made to maximize the information gained and the statistical rigor of the analysis while minimizing the number of systems analyzed. Four different experimental designs are presented by Donaghay (1984): single system, paired system, replicated, and single gradient. Each design has advantages and disadvantages, and each is appropriate for specific purposes.

4.13.6 Use of Data

Mesocosm research and microcosm research serve somewhat different purposes. If a researcher wants to have maximum confidence in extrapolating back to a specific, large-scale environment, the mesocosm provides greater realism in that it allows more large-scale processes to be included in the system. If simplification is the goal—either to semi-isolate certain components to determine their importance in the degradation of pollutants or to provide more easily analyzed ecosystems for test purposes—then microcosms are more appropriate.

The use of microcosms and/or mesocosms in an ocean discharge criteria evaluation as mandated under section 403 would be most appropriate for general permits issued for a whole category of discharges (e.g., regional oil and gas exploration/production activities) mainly because of the higher cost associated with implementing a series of such experiments. However, the use of microcosms and mesocosms can be less expensive than implementing field experiments and can provide quantitative estimates of processes and responses that can only be assessed qualitatively with field experiments. In addition, Perez and Morrison (1985) showed that the monetary costs of environmental

assessments of a single chemical using a single microcosm system versus a series of simple bioassay and physicochemical test systems are, at the worst case, effectively equivalent. Assessments of complex effluents performed using microcosms could result in more accurate results with a potential cost savings.

The use of microcosms and/or mesocosms could add significant benefits to an ecological risk assessment of an ocean discharge. Microcosms and/or mesocosms can be used to estimate ecological effects, chemical fate, transport mechanisms, bioaccumulation, and ecological risk. Where multiple discharges occur in one area, microcosms and/or mesocosms could be used to assess the risk associated with individual discharges or combinations of discharges. Estimating ecosystem recovery through the use of mesocosms in ecological risk assessments is a recent application (Perez et al., 1990) and could provide important information concerning the occurrence of irreparable harm.

4.13.7 Summary and Recommendations

Rationale

- Mesocosms and microcosms can be used to gain a realistic characterization of ecosystem-level impacts of marine discharges subject to section 403(c).
- The transport and fate of pollutants to the complete ecosystems can be investigated in near natural conditions.

Monitoring Design Considerations

- A microcosm should be designed to represent a miniature ecosystem that responds quickly to perturbation.
- Most generic microcosms do not simulate a site-specific natural system, but function as very generalized simulations of a large class of ecosystems.
- The design of the microcosm allows the system to be defined in terms of its physical and temporal boundaries, its light and temperature regime, its water composition, the turbulence and turnover rate, the ratio of benthic surface area to seawater volume, the sediment characteristics, and the water flow rate over the sediment surface.
- The results of microcosm or mesocosm experiments are measured by the ecological effects on the biota of the system.
- Microcosm studies have been designed for 30 days to more than a year in duration.

Analytical Method

- During microcosm studies, effluent to be tested should be added on a gradient. A typical experimental design might consist of six replicates in each of four treatment groups: a control, a single addition of a low concentration, a single addition of a high concentration, and repeated additions of low concentrations.
- Mesocosm systems can be classified by their degree of planktonic/benthic coupling into single, well-mixed benthic coupled; single stratified; and totally benthic decoupled systems.

QA/QC Analysis

- The results of microcosm and mesocosm studies are limited by the size of the system.
- Scaling laws may be used to translate from microcosms to full-scale ecosystems.

Statistical Design Considerations

- A multivariate analysis of variance, followed by univariate analysis, and regression techniques are recommended for the analysis of all data.
- Efforts must be made to maximize the information gained and the statistical rigor of the analysis while minimizing the number of systems analyzed.

Use of Data

- Data can be used to estimate ecological effects, chemical fate, transport mechanisms, bioaccumulation, and ecological risks.
- Data can also be used to assess impacts of individual or multiple discharges and to estimate recovery time.
- Mesocosms are most appropriate for use when maximum confidence in extrapolating back to a specific, large-scale environment is desired.
- Microcosms are most appropriate for use when simplification is the goal, either to isolate certain components to determine their importance or to provide more easily analyzed ecosystems for test purposes.
- Microcosms and mesocosms may be most appropriate for use for general permits issued for a whole category of discharges.
- There can be cost advantages to using microcosms and mesocosms under certain circumstances.

5. LITERATURE CITED

Adamus, P.R., E.J. Clairain, Jr., D.R. Smith, and R.E. Young. 1987. *Wetland evaluation technique (WET). Vol. II. Technical report Y-87.* U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS.

Anderberg, M. R. 1973. *Cluster analysis for applications.* Academic Press, New York, NY.

Anderson, D. 1990. Immunological indicators: Effects of environmental stress on immune protection and disease outbreaks. *Proceedings American Fisheries Society Symposium* 8: 38-50, Washington, DC.

Anderson, D., O. Dixon, and E. Lizzio. 1986. Immunization and culture of Rainbow Trout organ sections *in vitro. Vet. Immunol. Immun.* 12: 203-211.

Anderson, R.S. 1987. Immunocompetence in invertebrates. In *Pollutant studies in marine animals*, ed. C. S. Giam and L. E. Ray. CRC Press, Boca Raton, FL.

Anderson, R.S. 1990. Effects of pollutant exposure on bactericidal activity of *Mercenaria mercenaria* hemolymph. In *Biological markers of environmental contaminants*, ed. J.F. McCarthy and L.R. Shugart. American Chemical Society, Los Angeles, CA.

APHA. 1989. American Public Health Association, American Water Works Association, and Water Pollution Control Federation. *Standard methods for the examination of water and wastewater.* 17th ed. American Public Health Association, Washington, DC.

Appell, G.F., and T.B. Curtin. 1990. *Proceedings of the IEEE Fourth Working Conference on Current Measurement.* Institute of Electrical and Electronic Engineers, New York, NY.

Appell, G.F., and W.E. Woodward. 1986. *Proceedings of the IEEE Third Working Conference on Current Measurement.* Institute of Electrical and Electronic Engineers, New York, NY.

Armstrong, N.E., P.N. Storrs, and H.F. Ludwig. 1970. Ecosystem-pollution interactions in San Francisco Bay. *J. Water Poll. Control Fed.*

ASTM. 1990. *Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods.* Guide E1367-90. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1991. Standard guide for collection, storage, characterization, and manipulation of sediments for toxicological testing. ASTM Designation E1391-90. In *Annual book of ASTM standards.* American Society for Testing and Materials, Philadelphia, PA.

Azam, F., T. Fenchel, J.G. Field, J.S. Gray, L.A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water column microbes in the sea. *Mar. Ecol. Prog. Ser.* 10:257-263.

Bechtel, T.J., and B.J. Copeland. 1970. Fish species diversity indices as indicators of pollution in Galveston Bay, Texas. *Contrib. in Mar. Sci.* 15:103-132.

Becker, D.S., and J. W. Armstrong. 1988. Development of regionally standardized protocols for marine environmental studies. *Mar. Pollut. Bull.* 19(7):310-313.

Beers, J.R., G.L. Stewart, and J.D.H. Strickland. 1967. A pumping system for sampling small plankton. *J. Fish. Res. Board Can.* 24:1811-1818.

Beukema, J.J. 1988. An evaluation of the ABC method (abundance-biomass comparison) as applied to macrozoobenthic communities living on tidal flats in the Dutch Wadden Sea. *Mar. Biol.* 99: 425-433.

Bilyard, G.R. 1987. The value of benthic infauna in marine pollution monitoring studies. *Mar. Poll. Bull.* 18: 581-585.

Bisson, J.W., and V.J. Cabelli. 1979. Membrane filtration enumeration method for *Clostridium perfringens. Appl. Environ. Microbiol.* 37: 55-66.

Blaxhall, P., and K. Daisley. 1973. Routine haematological methods for use with fish blood. *J. Fish Biol.* 5: 771.

Boehm, P.D. 1984. *The Status and Trends Program: Recommendations for design and implementation of the chemical measurement segment.* Workshop report. NOAA, Rockville, MD.

Boesch, D. F. 1977. *Application of numerical classification in ecological investigations of water pollution.* EPA 600/3-77-033. U.S. Environmental Protection Agency, Office of Research and Development, Corvallis, OR.

Bohnsack, J. A. 1979. Photographic quantitative sampling of hard-bottom benthic communities. *Bull. Mar. Sci.* 29:242-252.

Bond, C.E. 1979. *Biology of fishes.* Sanders College Publishing, Philadelphia, PA.

Bordner, R.H., J.A Winter, and P.V Scarpino, eds. 1978. *Microbiological methods for monitoring the environment, water and waste.* EPA/600/8-78-017. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

Borrego, JJ., F. Arrabal, A. de Vicente, L.F. Gomez, and P. Romero. 1983. Study of microbial inactivation in the marine environment. *J. Water Pollut. Control Fed.* 55: 297-302.

Bouck, G.R. 1984. Physiological responses of fish: Problems and progress toward use in environmental monitoring. In *Contaminant effects on fisheries*, ed. V. W. Cairns, P. V. Hodson, and J. O. Nriagu. John Wiley and Sons, New York, NY.

Brainard, E.C., and R.J. Lukens. 1975. *A comparison of the accuracies of various continuous recording current meters for offshore use.* Offshore technology conference, Paper no. OTC 2295.

Bros, W.E., and B.C. Cowell. 1987. A technique for optimizing sample size (replication). *J. Exp. Mar. Biol. Ecol.* 30: 21-35.

Brown, B. E. 1988. Assessing environmental impacts on coral reefs. *Proc. 6th Int. Coral Reef Symp.* 1:71-80.

Brusick, D. 1980. *Principles of genetic toxicology.* Plenum Press, New York, NY.

Buckley, L.J., T.A. Halavik, G.C. Lawrence, S.J. Hamilton, and P. Yevich. 1985. Comparative swimming stamina, biochemical composition, backbone mechanical properties, and histopathology of juvenile striped bass from rivers and hatcheries of the eastern United States. *Trans. Amer. Fish. Soc.* 114:114-124.

Buller, A.T., and J. McManus. 1979. Sediment sampling and analysis. In *Estuarine hydrography and sedimentation*, ed. K.R. Dyer. Cambridge University Press, Cambridge.

Cabelli, V.J., A.P. DuFour, M.A. Levin, L.J. McCabe, and P.W. Haberman. 1979. Relationship of microbial indicators to health effects at marine bathing beaches. *Am. J. Public Health* 69: 690-696.

Cabelli, V.J., A.P. DuFour, L.J. McCabe, and M.A. Levin. 1982. Swimming-associated gastroenteritis and water quality. *Am. J. Epidemiol.* 115: 606-61.

Cabelli, V.J., A.P. DuFour, L.J. McCabe, and M.A. Levin. 1983. A marine recreational water quality criterion consistent with indicator concepts and risk analysis. *J. Water Pollut. Control Fed.* 55: 1306-1314.

Cailliet, G.M., M.S. Love, and A.W. Ebeling. 1986. *Fishes: A field and laboratory manual on their structure, identification, and natural history.* Wadsworth Publishing Company, Belmont, CA.

CDC. 1979. Viral hepatitis outbreaks-Georgia, Alabama. Centers for Disease Control. *Morbid. Mortal. Weekly Rep.* 28: 581.

Chesapeake Executive Council. 1988a. *Habitat requirements for Chesapeake Bay living resources.* Chesapeake Bay Program, Annapolis, MD.

Chesapeake Executive Council. 1988b. *Living resources monitoring plan.* Chesapeake Bay Program, Annapolis, MD.

Clausner, J.E., W.A. Birkemeier, and G.R. Clarke. 1986. *Field comparison of four nearshore survey systems.* Miscellaneous Paper CERC-86-6. U.S. Army Engineer Waterways Experimental Station, Vicksburg, MS.

Clifford, H.T., and W. Stephenson. 1975. *An introduction to numerical classification.* Academic Press, New York, NY.

Connell, J.H. 1978. Diversity in tropical rain forests and coral reefs. *Science* 199: 1302-1309.

Copeland, B.J. 1966. Effects of industrial waste on the marine environment. *J. Water Poll. Cont. Fed.* 38: 1000-1010.

Copeland, B.J., and D.E. Wohlschlag. 1968. Biological responses to nutrients-eutrophication: Saline water considerations In *Advances in water quality improvement,* ed. E.F. Gloyna and W.W. Eckenfelder, pp. 65-82. Univ. Tex. Press, Austin, TX.

Couch, J.A. 1978. Diseases, parasites, and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic coasts of North America. *Fish. Bull.* 76: 1-44.

Couch, J.A., and J.W. Fournie. 1993. Pathobiology of marine and estuarine organisms. In *Advances in fisheries science.* CRC Press, Boca Raton, FL.

Couch, J.A., and J.C. Harshbarger. 1985. Effects of carcinogenic agents on aquatic animals: An environmental and experimental overview. *Environ. Carcinogenesis Revs.* 3(1): 63-105.

Cuff, W., and N. Coleman. 1979. Optimal survey design: Lessons from a stratified random sample of macrobenthos. *J. Fish. Res. Board Can.* 36: 351-361.

Curtis, M.A., and G.H. Peterson. 1978. Size-class heterogeneity with spatial distribution of subarctic marine benthos populations. *Astarte* 10: 103-105.

Cushing, D.J. 1975. *Marine ecology and fisheries.* Cambridge University Press, Cambridge, UK.

Darcy, G. H. and E. J. Gutherz. 1984. Abundance of demersal fishes on the west Florida shelf, January 1978. *Bull. Mar. Sci.* 34:81-105.

Davis, R.E. 1988. Modeling eddy transport of passive tracers. *J. Mar. Res.* 45: 635.

Dawe, C.J., J.C. Harshbarger, R. Wellings, and J. D. Strandberg. In press. *The pathobiology of spontaneous and induced neoplasms in fishes: Comparative characterization, nomenclature, and literature.* Academic Press, New York, NY.

Day, J.W., C.A.S. Hall, W.M. Kemp, and A. Yanez-Arancibia. 1989. *Estuarine ecology.* John Wiley and Sons, New York, NY.

Dayton, P. K. 1985. The structure and regulation of some South American kelp communities. *Ecol. Monagr.* 55(4):447-468.

deBoer, J. 1988. Chlorobiphenyls in bound and non-bound lipids of fishes: Comparison of different extraction methods. *Chemosphere* 17: 1803-1810.

D'Elia, C.F., J.G. Sanders, and D.G. Capone. 1989. Analytical chemistry for environmental sciences: A question of confidence. *Environ. Sci. Technol.* 23(7):768-774.

D'Elia, C.F., K.L. Webb, D.V. Shaw, and C.W. Keefe. 1986. *Methodological comparisons for nitrogen and chlorophyll determinations in estuarine water samples.* Submitted to the Power Plant Siting Program, Maryland Department of Natural Resources, Annapolis, MD, and Chesapeake Bay Liaison Office, U.S. Environmental Protection Agency, Annapolis, MD.

Demmann, W.P., J.R. Proni, J.F. Craynock, and R. Fargen. In press. *Oceanic wastewater outfall plume characterization measured acoustically.*

DiToro, D.M., J.D. Mahony, D.J. Hansen, K.J. Scott, A.R. Carlson, and G.T. Ankley. In press a. *Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments.*

DiToro, D.M., J.D. Mahony, D.J. Hansen, K.J. Scott, M.B. Hicks, S.M. Mayr, and M.S. Redmond. In press b. *Toxicity of cadmium in sediments: The role of acid volatile sulfide.*

Dodge, R. E., A. Logan, and A. Antonius. 1982. Quantitative reef assessment studies in Bermuda: A comparison of methods and preliminary results. *Bull. Mar. Sci.* 32(3):745-760.

Donaghay, P.L. 1984. Utility of mesocosms to assess marine pollution. In *Concepts in marine pollution measurements*, ed. H.H. White, pp. 589-620. Maryland Sea Grant College, College Park, MD.

Downing, J.A. 1979. Aggregation, transformation, and the design of benthos sampling programs. *J. Fish Res. Board Cer.* 36:1454-1463.

DuFour, A.P., E.R. Strickland, and V.J. Cabelli. 1981. Membrane filter method for enumerating *Escherichia coli. Appl. Environ. Microbiol.* 41: 1152-1158.

Dustan, P., and J. C. Halas. 1987. Changes in the reef-coral community of Carysfort Reef, Key Largo, Florida: 1974 to 1982. *Coral Reefs* 6(2):91-106.

EA and Battelle. 1990. *Evaluation of dredged material proposed for ocean disposal (testing manual).* Draft report prepared by EA Engineering, Science, and Technology, Inc. and Battelle Ocean Sciences for the U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

Eganhouse, R.P. 1990. Sources and magnitude of error associated with PCM measurements. In *Southern California Coastal Water Research Project annual report 1989-1990*, ed. J.N. Cross and D.M. Wiley. Southern California Coastal Water Research Project, Long Beach, CA.

Eleftheriou, A., and N.A. Holme. 1984. Macrofauna techniques. In *Methods for the study of marine benthos*, ed. N.A. Holmes and A.D. McIntyre, pp. 66-98. Blackwell Scientific Publications, Oxford.

Elliot, J.M. 1971. *Some methods for the statistical analysis of samples of benthic invertebrates.* Scientific Publication No. 25, Freshwater Biological Assn., Ferry House, UK.

Ellis, A. 1977. The leucocytes of fish: A review. *J. Fish Biol.* 11:453.

Ellis, D. 1985. Taxonomic sufficiency in pollution assessment. *Mar. Poll. Bull.* 16: 459.

Emerson, D.J., and V.J. Cabelli. 1982. Extraction of *Clostridium perfingens* spores from bottom sediment samplers. *Appl. Environ. Microbiol.* 44: 1152-1158.

Engel, D., and G. Roesijadi. 1987. Metallothioneins: A monitoring tool. In *Pollution physiology of estuarine organisms*, ed. W. Vernberg, A. Calabrese, F. Thurberg, and F. J. Vernberg, pp. 421-438. University of South Carolina Press.

Eugene, L.B. 1966. *Handbook of oceanographic tables.* Naval Oceanographic Office, Rep. SP-68.

Feingold, A.O. 1973. Hepatitis from eating steamed clams. *J. Am. Med. Assoc.* 225: 526-527.

Ferguson, H.W. 1989. *Systemic pathology of fish: A text and atlas of comparative tissue responses in diseases of teleosts.* Iowa State University Press, Ames, IA.

Ferraro, S.P., F.A. Cole, W.A. DeBen, and R.C. Swartz. 1989. Power-cost efficiency of eight macrobenthic sampling schemes in Puget Sound, WA, USA. *Can. J. Fish. Aquat. Sci.* 46: 2157-2165.

Ferraro, S.P., H. Lee, R.J. Ozretich, and D.T. Specht. 1990. Predicting bioaccumulation potential: A test of a fugacity-based model. *Arch. Environ. Contam. Toxicol.* 19:386-394.

Fleishack, P.C., A.J. DeFreitas, and R.B. Jackson. 1985. Two apparatuses for sampling benthic fauna in surf zones. *Est. Coast. Shelf Sci.* 21:287-293.

Fofonoff, N.P., and R.C. Millard, Jr. 1983. *Algorithms for computation of fundamental properties of seawater.* Technical papers in marine science no. 44. United Nations Educational, Scientific, and Cultural Organization (UNESCO), Paris, France.

Folk, R.L. 1974. *Petrology of sedimentary rocks.* Herpmill Publishing Co., Austin, TX.

Folmar, L.C. 1993. Effects of chemical contaminants on blood chemistry of teleost fish: A bibliography and synopis of selected effects. *Environ. Toxicol. Chem.* 12:337-375.

Fowler, S.W. 1982. Biological transfer and transport processes. In *Pollutant transfer and transport in the sea*, vol. 2, ed. G. Kullenberg. CRC Press, Boca Raton, FL.

Fredette, T.J., D.A. Nelson, T. Miller-Way, J.A. Adair, V.A. Sotler, J.E. Clausner, E.B. Hands, and F.J. Anders. 1989. *Selected tools and techniques for physical and biological monitoring of aquatic dredged material disposal sites.* Final report. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Gamble, J. C. 1984. Diving. In *Methods for the study of marine benthos*, ed. N.A. Holmes and A. D. McIntyre, pp. 66-68. Blackwell Scientific Publications, Oxford.

Gameson, A.L.N. 1983. Investigation of sewage discharges to some British coastal waters. Water Resources Centre Technical Report, TR 193. Bucks, United Kingdom.

Gardner, G.R., P.P. Yevich, J.C. Harshbarger, and A.R. Malcolm. 1991. Carcinogenicity of Black Rock Harbor sediment to the eastern oyster and trophic transfer of Black Rock Harbor carcinogens from the blue mussel to the winter flounder. *Environ. Health Perspect.* 90: 53-66.

Gardner, W.S., T.F. Nalepa, W.A. Frez, E.A. Cichocki, and P.F. Landrum. 1985. Seasonal patterns in lipid content of Lake Michigan macroinvertebrates. *Can. J. Fish. Aquat. Sci.* 42: 1827-1832.

Garvey, J. 1990. Metallothionein: A potential biomarker of exposure to environmental toxins. In *Biomarkers of environmental contamination*, ed. J. McCarthy and L. Shugart. CRC Press, Boca Raton, FL.

Geldreich, E.E., P.W. Kabler, H.L. Jeter, and H.F. Clark. 1955. A delayed incubation membrane filter test for coliform bacteria in water. *Ameri. J. Pub. Health* 45: 1462.

Gilliom, R.J., and D.R. Helsel. 1986. Estimation of distributional parameters for censored trace level water quality data. 1, Estimation Techniques. *Water Res. Res.* 22(2): 135-146.

Goldberg, E.D., V.T. Bowen, G.H. Farrington, J.H. Martin, P.L. Parker, R.W. Risebrough, W. Robertson, E. Schneider and E. Gamble. 1978. The mussel watch. *Environ. Conserv.* 5:101-125.

Grace, R.A. 1978. *Marine outfall systems planning, design, and construction.* Prentice Hall, Inc., Englewood Cliffs, NJ.

Gray, J.S., and F.B. Mirza. 1979. A possible method for the detection of pollution induced disturbance on marine benthic communities. *Mar. Poll. Bull.* 10: 142-146.

Green, R. 1979. *Sampling design and statistical methods for environmental biologists.* John Wiley and Sons, New York, NY.

Green, R.H. 1984a. Some guidelines for the design of biological monitoring programs in the marine environment. In *Concepts of marine pollution measurements*, ed. H.H. White, pp. 233-245. University of Maryland Sea Grant, College Park, MD.

Green, R.H. 1984b. Statistical and nonstatistical considerations for environmental monitoring studies. *Environ. Monit. Assess.* 4: 293-301.

Grigg, R. W., and S. J. Dollar. 1990. Natural and anthropogenic disturbance on coral reefs. In *Coral reefs*, ed. Z. Dubinsky, pp. 439-452. Ecosystems of the world 25. Elsevier, New York, NY.

Hansen, P.D., H. Von Westerhagen, and H. Rosenthal. 1985. Chlorinated hydrocarbons and hatching success in Baltic herring spring spawners. *Mar. Environ. Res.* 15: 59-76.

Hardy, J.T. 1982. The sea surface microlayer: Biology, chemistry, and anthropogenic enrichment. *Prog. Oceanogr.* 11: 307-328.

Hargis, W., M. Roberts, and D. Zwerner. 1984. Effects of contaminated sediments and sediment-exposed effluent water on estuarine fish: Acute toxicity. *Mar. Environ. Res.* 14: 337-354.

Hargis. W., and D. Zwerner. 1988. Effects of certain contaminants on eyes of several estuarine fishes. *Mar. Environ. Res.* 24: 265-270.

Haux, C., and L. Förlin. 1988. *Biochemical methods for detecting effects of contaminants on fish.*

Heath, A. 1987. *Water pollution and fish physiology.* CRC Press, Boca Raton, FL.

Hiatt, M.H. 1981. Analysis of fish and sediment for volatile priority pollutants. *Anal. Chem.* 53: 1541-1543.

Hill K.D., T.M. Dauphinee, and D. J. Woods. 1986. Extension of the practical salinity scale 1978 to low salinities. *IEEE Journal of Oceanic Engineering* OE-11 (1, January 1986): 109-112.

Hinton, D., J. Couch, S. Teh, and L. Courtney. 1988. Cytological changes during progression of neoplasia in selected fish species. In *Aquatic life toxicology, toxic chemicals and aquatic life: Research and management*, ed. D. Malins, A. Jensen, and M. Moore. Elsevier Science Publishers.

Hinton, D.E., and J.A. Couch. 1984. Pathobiological measures of marine pollution effects. In *Concepts in marine pollution measurements*, ed. H.H. White, pp. 7-32. Maryland Sea Grant College, College Park, MD.

Hirsch, R.M. 1988. Statistical methods and sampling design for estimating step trends in surface-water quality. *Water Res. Bull.* 24(3): 493-503.

Holme, N. A. 1984. Photography and television. IBP handbook no. 16. In *Methods for the study of marine benthos*, ed. N. A. Holmes and A. D. McIntyre, pp. 66-98. Blackwell Scientific Publications, Oxford.

Holme, N.A., and A.D. McIntyre, eds. 1984. *Methods for the study of marine benthos.* Blackwell Scientific Publications, Oxford.

Howard, D.W., and C.S. Smith. 1983. *Histological techniques for marine bivalve molluscs.* NOAA Technical Memorandum. NMFS-F/NEC-25. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Woods Hole, MA.

Hunn, J. 1988. *Field assessment of the effects of contaminants on fishes.* Biological Report 88, Fish and Wildlife Service, U.S. Department of the Interior, Columbia, MO.

Hurlbert, S.H. 1971. The nonconcept of species diversity: A critique and alternative parameters. *Ecology* 52: 577-586.

Inhorn, S.L., ed. 1977. *Quality assurance practices for health laboratories.* American Public Health Assoc., Washington, DC.

Jacobs, F., and G.C. Grant. 1978. *Guidelines for zooplankton sampling in quantitative baseline and monitoring programs.* Rep. No. 600/3-7-78-026. U.S. Environmental Protection Agency, Corvallis, OR.

Jimenez, B., A. Oikari, S. Adams, D. Hinton, and J. McCarthy. 1990. Hepatic enzymes as biomarkers of environmental, physiological and toxicological variables. In *Biomarkers of environmental contamination*, ed. J. McCarthy and L. Shugart, pp. 123-142. CRC Press, Boca Raton, FL.

Johnson, R., and H. Bergman. 1984. Use of histopathology in aquatic toxicology: A critique. In *Contaminant effects on fisheries*, ed. V. Cairns, P. Hodson, J. Nriagu. John Wiley and Sons, New York, NY.

Kaplan, J.E., R.A. Goodman, L.B. Schonberger, E.C. Lippy, and G.W. Gary. 1982. Gastroenteritis due to Norwalk virus: An outbreak association with municipal water system. *J. Infect. Dis.* 146: 190-197.

Karickhoff, S.W., D.S. Brown, and T.A. Scott. 1979. Sorption of hydrophobic pollutants on natural sediments. *Wat. Res.* 13: 241-248.

Karr, J.R., et al. 1986. *Assessing biological integrity in running waters: A method and its rationale.* Ill. Nat. Hist. Survey Special Publ. 5.

Kleinow, K., M. MeLancon, and J. Lech. 1987. Biotransformation and induction: Implications for toxicity, bioaccumulation and monitoring of environmental xenobiotics in fish. *Environ. Health Perspect.* 71: 105-119.

Klingerman, A.D. 1982. Fishes as biological detectors of the effects of genotoxic agents. In *Mutagenicity, new horizons in genetic toxicology*, ed. J. Heddle. Academic Press.

Klontz, G. 1985. Diagnostic methods in fish diseases: Present status and needs. In *Fish and shellfish pathology*, ed. A. Ellis. Academic Press Inc.

Knezovich, J.P., and F.L. Harrison. 1987. A new method for determining the concentration of volatile organic compounds in sediment interstitial water. *Bull. Environ. Contam. Toxicol.* 38: 837-940.

Koh, R.C.Y. 1988. Shoreline impact from ocean waste discharges. *J. Hydraul. Div. Am. Soc. Civ. Eng.* 114:361.

Ladd, J.M., S.P. Hayes, M. Martin, M.D. Stephenson, S.L. Coale, J. Linfield, and M. Brown. 1984. *California state mussel watch: 1981-1983. Trace metals and synthetic organic compounds in mussels from California's coast, bays and estuaries.* Biennial report. Water Quality Monitoring Report No. 83-6TS. Sacramento, CA.

Lake, J.L., N.I. Rubenstein, and S. Parvigano. 1987. Predicting bioaccumulation: Development of a partitioning model for use as a screen tool in regulating ocean disposal of wastes. In *Fate and effects of sediment-bound chemicals in aquatic systems,* ed. K.L. Dickson, A.W. Maki, and W.A. Brungo. Sixth Pellston Workshop, Florissano, CO.

Landolt, M.L., and R.M. Kocan. 1983. Fish cell cytogenetics: A measure of the genotoxic effects of environmental pollutants. In *Aquatic toxicology*, ed. J. Nriagu. John Wiley and Sons, New York, NY.

Larson, A., C. Haux, and M. Sjobeck. 1985. Fish physiology and metal pollution: Results and experiences from laboratory and field studies. *Ecotoxicol. Environ. Saf.* 9: 250.

Lech, J., M. Vodicnik, and C. Elcombe. 1982. Induction of mono-oxygenase activity in fish. In *Aquatic toxicology*, ed. L. Weber, pp. 107-148. Raven Press.

Leighton, J.P., et al. 1988. Verification/Calibration of a thermal discharge model. *Proc. Int. Symp. Model-Prototype Correlation of Hydraul. Structures* 148.

Lenat, D.R. 1988. Water quality assessments of streams using a qualitative collection method for benthic macroinvertebrates. *J.N. Am. Benthol. Soc.* 7: 222.

Levin, M.A., J.R. Fischer, and V.J. Cabelli. 1975. Membrane filter technique for enumeration of enterococci in marine waters. *Appl. Microbiol.* 30: 66.

Livingston, R.J., R.S. Lloyd, and M.S. Zimmerman. 1976. Determination of sampling strategy for benthic macrophytes in polluted and unpolluted coastal areas. *Bull. Mar. Sci.* 26: 569-575.

Lonard, R.I., and E.J. Clairain, Jr. 1986. Identification of methodologies for the assessment of wetland functions and values, In *Proceedings: National wetlands assessment symposium,* pp. 66-72. Association of State Wetland Managers, Inc., Portland, ME, June 17-20.

Lorenzen, C.J. 1966. A method for the continuous measurement of in vivo chlorophyll concentration. *Deep-Sea Res.* 13: 223-227.

Loya, Y. 1978. Plotless and transect methods. In *Coral reefs: Research methods.*, ed. D.R. Stoddart and R.E. Johannes, pp. 197-217. UNESCO, Monographs on Oceanographic Methodology, Paris.

Maine DEP. 1987. *Methods for biological sampling and analysis of Maine's waters.* Maine Bureau of Water Quality Control.

Margalef, R. 1969. Diversity and stability: A practical proposal and a model of interdependence. In *Diversity and stability in ecological systems*, ed. G.M. Woodwell and H.H. Smith. *Brookhaven Symp. Biol.* 22: 25-37.

Matthews, E., J. Warinner, and B. Weeks. 1990. Assays of immune function in fish macrophages. In *Techniques in fish immunology*, ed. J. Stolen, T. Fletcher, D. Anderson, B. Roberson, and W. van Muiswinkel. SOS Publications, Fair Haven, NJ.

McCarthy, J.F. 1990. Concluding remarks: Implementation of a biomarker-based environmental monitoring program. In *Biomarkers of environmental contamination*, ed. J. McCarthy and L. Shugart. Lewis Publishers, Boca Raton, FL.

McCullough, J.R. 1977. Problems in measuring currents near the ocean surface. *Proceedings of Oceans* '77, Marine Tech. Soc. and Inst. of Electrical and Electronics Engineering.

McFarland, V.A., J.U. Clarke, and A.B. Gibson. 1986. *Changing concepts and improved methods for evaluating the importance of PCBs as dredged sediment contaminants.* Miscellaneous Paper D-86-5. Department of the Army, Corps of Engineers, Waterways Experiment Station, Vicksburg, MS.

McGowan, J.A., and V.J. Fraundorf. 1966. The relationship between size of net used and estimates of zooplankton diversity. *Limnol. Oceanogr.* 11: 456-469.

McIntyre, A.D., J.M. Elliot, and D.V. Ellis. 1984. Introduction: Design of sampling programs. IBP Handbook No. 16. In *Methods for the study of marine benthos*, ed. N.A. Holme and A.D. McIntyre, pp. 1-26. Blackwell Scientific Publications, Oxford.

Meyers, T.R., and J.D. Hendricks. 1985. Histopathology. In *Fundamentals of aquatic toxicology: Methods and applications*, ed. G. M. Rand and S. R. Petrocelli, pp. 283-331. Hemisphere Publishing Co., New York, NY.

Millard, S.P., and D.P. Lettenmaier. 1986. Optimal design of biological sampling programs using the analysis of variance. *Est. Coast. Shelf Sci.* 22: 637-656.

Mix, M.C. 1986. Cancerous diseases in aquatic animals and their association with environmental pollutants: A critical literature review. *Mar. Environ. Res.* 20 (1&2):1-141.

Neff, J.M. 1985. *Use of biochemical measurements to detect pollutant-mediated damage to fish.* American Society for Testing and Materials, Special Technical Publication 854: 155-181.

Nielson, L.A., and D.L. Johnson, eds. 1984. *Fisheries techniques.* American Fisheries Society, Bethesda, MD.

Niimi, A.J. 1983. Biological and toxicological effects of environmental contaminants in fish and their eggs. *Can. J. Fish. Aquat. Sci.* 40: 306-312.

NOAA. 1988. *National Marine Pollution Program federal plan for ocean pollution research, development and monitoring.* U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Washington, DC.

NRC. 1990. *Managing troubled waters: The role of marine environmental monitoring.* National Research Council. National Academy Press, Washington, DC.

Odum, H.T., R.P. Cuzan du Rest, R.J. Beyers, and C. Allbaugh. 1963. Diurnal metabolism, total phosphorus, Ohle anomaly, and zooplankton diversity of abnormal marine ecosystems of Texas. *Publs. Inst. Mar. Sci. Tex.* 9: 404-453.

Ohara, H., H. Naruto, W. Watanabe, and I. Ebisawa. 1983. An outbreak of Hepatitis A caused by consumption of raw oysters. *J. Hyg. Camb.* 91: 163-165.

Ohio EPA. 1987. *Biological criteria for the protection of aquatic* life: Vols. 1, 2, and 3. Division of Water Quality Monitoring and Assessment. Columbus, OH.

Ohlhorst, S.L., W.D. Liddell, R.J. Taylor, and J.M. Taylor. 1988. Evaluation of reef census techniques. *Proc. 6th Int. Coral Reef Symp.* 2:319-324.

OTA. 1987. *Wastes in marine environments.* U.S. Congress, Office of Technology Assessment. OTA 0-334.

Patten, B.C. 1962. Species diversity in net phytoplankton of Raritan Bay. *J. Mar. Res.* 20: 57-75.

Payne, J., L. Fancey, A. Rahimtula, and E. Porter. 1987. Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. *Comp. Biochem. Physiol.* C86: 233-245.

Pearson, T.H., and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceangr. Mar. Biol. Ann. Rev.* 16: 229-311.

Pederson, D.C. 1980. *Density levels of pathogenic organisms in municipal wastewater sludges: A literature review.* Prepared by Camp Dresser and McKee, Inc. for U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.

Perez, K.T., E.W. Davey, J. Heltsche, J.A. Cardin, N.F. Lackie, R.L. Johnson, R.J. Blasco, A.E. Soper, and E. Read. 1990. *Recovery of Narragansett Bay, RI: A feasibility study.* ERLN Contribution No. 1148. U.S. Environmental Protection Agency, Environmental Research Laboratory, Ecosystems Effects Branch, Narragansett, RI.

Perez, K.T., and G.E. Morrison. 1985. Environmental assessments from simple test systems and a microcosm: Comparisons of monetary costs. In *Multispecies toxicity testing*, ed. J. Cairns, pp. 89-95.

Perez, K.T., G.E. Morrison, E.W. Davey, N.F. Lackie, A.E. Soper, R.J. Blasco, D.L. Winslow, R.L. Johnson, P.G. Murphy, J.F. Heltsche. In press. *Influence of size on the fate and ecological effects of the pesticide kepone in a physical simulation model.* U.S. Environmental Protection Agency, Environmental Research Laboratory, Ecosystems Effects Branch, Narragansett, RI.

Peters, G., H. Delventhal, and H. Klinger. 1980. Physiological and morphological effects of social stress in the eel, *Anguilla anguilla. L. Art. Fish. Wiss.* 30: 157.

Phillips, D.J.H. 1980. *Quantitative aquatic biological indicators.* Applied Science Publ. Ltd., London, England.

Phillips, D.J.H., and D.S. Segar. 1986. Use of bio-indicators in monitoring conservative contaminants: Programme design imperatives. *Mar. Poll. Bull.* 17: 10-17.

Phillips, N.W., D.A. Gettleson, and K.D. Spring. 1990. Benthic biological studies of the southwest Florida shelf. *Amer. Zool.* 30(1):65-75.

Pickard, G.L., and W.J. Emery. 1982. *Descriptive physical oceanography, An introduction.* 4th (SI) enlarged ed. Pergamon Press, Inc., New York, NY.

Pickering, A. 1981. *Stress and fish.* Academic Press.

Pielou, E.C. 1966. The measurement of diversity in different types of biological collections. *J. Theoret. Biol.* 13: 131-144.

Pielou, E.C. 1984. *The interpretation of ecological data.* John Wiley and Sons, New York, NY.

Plafkin, J.L., et. al. 1989. *Rapid bioassessment protocols for use in streams and rivers.* Office of Water Regulations and Standards. EPA 444/4-89-001.

Plumb, R.H., Jr. 1981. *Procedure for handling and chemical analysis of sediment and water samples.* Technical report EPA/CE-81-1. Prepared for the U.S. Environmental Protection Agency/Corps of Engineers Technical Committee on Criteria for Dredged and Fill Material, by Great Lakes Laboratory, State University College at Buffalo, Buffalo, NY. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Pomeroy, L.R. 1984. Significance of microorganisms in carbon and energy flow in marine ecosystems. In *Current perspectives in microbial ecology*, ed. M.J. Klug and C. A. Reddy. pp. 405-411. American Society for Microbiology, Washington, DC.

Pond, S., and G.L. Pickard. 1983. *Introductory dynamic oceanography.* 3d ed. Pergamon Press, Inc., New York, NY.

Pontasch, K.W., B.R. Niederlehner, and J. Cairns, Jr. 1989. Comparisons of single-species microcosm and field responses to a complex effluent. *Environ. Tox. and Chem.* 8: 521-532.

Porter, J.W. 1972. Patterns of species diversity in Caribbean reef corals. *Ecology* 53: 745-748.

Porter, P.S., R.C. Ward, and H.F. Bell. 1988. The detection limit. *Env. Sci. Tech.* 22: 856-861.

Prophet, E.B., B. Mills, J.B. Harrington, and L.H. Sorbin. 1992. *AFIP laboratory methods in histotechnology.* Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC.

Raffaelli, D., and C.F. Mason. 1981. Pollution monitoring with meiofauna, using the ratio of nematodes to copepods. *Mar. Poll. Bull.* 12: 158-163.

Rees, H.L. 1984. A note on mesh selection and sampling efficiency in benthic studies. *Mar. Pollut. Bull.* 15: 225-229.

Reish, D.J. 1959. A discussion of the importance of screen size in washing quantitative marine bottom samples. *Ecology* 40: 307-309.

Reish, D.J. 1986. Benthic invertebrates as indicators of marine pollution: 35 years of study. *IEEE Conference Proceedings*, Oceans '86, pp. 885-888.

Rhoads, D.C., and J.D. Germano. 1982. Interpreting long-term changes in benthic community structure: A new protocol. *Hydrobiologia* 142: 291-308.

Rhoads, D.C., and J.D. Germano. 1986. Characterization of organism-sediment relations using sediment profile imaging: An efficient method of remote ecological monitoring of the sea floor (REMOTS system). *Mar. Ecol. Prog.* Ser. 8: 115-128.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. *Bull. Fish. Res. Bd. Can.* 191: 382 pp.

Roberts, R.J. 1989. *Fish pathology.* Ballmore Tindall, London, England.

Rogers, C.S. 1988. Recommendations for long-term assessment of coral reefs: U.S. National Park Service initiates regional program. *Proc. 6th Int. Coral Reef Symp.* 1: 399-403.

Rogers, C.S., M. Gilnack, and H.C. Fitz III. 1983. Monitoring of coral reefs with linear transects: a study of storm damage. *J. Exp. Mar. Biol. Ecol.* 49: 179-187.

Romesburg, H.C. 1984. *Cluster analysis for researchers.* Lifetime Learning Publications, Belmont, CA.

Rothschild, B.J. 1986. *Dynamics of marine fish populations.* Harvard University Press, Cambridge, MA.

Rowley, A. 1990. Collection, separation and identification of fish leucocytes. In *Techniques in fish immunology*, ed. J. Stolen, T. Fletcher, D. Anderson, B. Roberson, and W. van Muiswinkel. SOS Publications, Fair Haven, NJ.

Sanders, B. 1990. Stress proteins. In *Biomarkers of environmental contamination*, ed. J. McCarthy and L. Shugart. CRC Press, Boca Raton, FL.

SCCWRP. 1988. *Recovery of Santa Monica Bay after termination of sludge discharge.* 1988-1989 Annual Report, Southern California Coastal Water Research Project, pp. 46-53.

Schackleford, B. 1988. *Rapid bioassessments of macroinvertebrate communities: Biocriteria development.* Arkansas Dept. Poll. Contr. and Ecol., Little Rock, AR.

Shepard, F.P. 1954. Nomenclature based on sand-silt-clay ratios. *J. Sed. Petrol.* 24(3):151-158.

Sherr, B., and E. Sherr. 1983. Enumeration of heterotrophic microprotozoa by epifluorescence microscopy. *Est. Coast. Shelf Sci.* 16: 1-7.

Shugart, L.R. 1990. Biological monitoring: Testing for genotoxicity. In *Biomarkers of environmental contamination*, ed. J. McCarthy and L. Shugart. CRC Press, Boca Raton, FL.

Sindermann, C.J. 1990. *Principal diseases of marine fish and shellfish.* Vols. 1 and 2. Academic Press, San Diego, CA.

Sneath, P.H.A. and R.R. Sokal. 1973. *Numerical taxonomy: The principles and practices of numerical classification.* Freeman, San Francisco, CA.

Sokal, R.R., and F.J. Rohlf. 1981. *Biometry.* W.H. Freeman and Co., San Francisco.

Sorenson, E.M.B., and N.K.R. Smith. 1981. Hemosiderin granules: Cytotoxic response to arsenic exposure in channel catfish, *Ictalurns punctatus. Bull. Environ. Contam. Toxicol.* 27: 645-653.

Sparks, A.K. 1985. *Synopsis of invertebrate pathology exclusive of insects.* Elsevier Science Publishers, Amsterdam.

Spies, R.B., D.W. Rice, and J. Felton. 1988. Effects of organic contaminants on reproduction of the starry flounder *Platichthys stellatus* in San Francisco Bay. *Mar. Biol.* 98: 181-189.

St. John, E.W., J.R. Matches, and M.M. Wekell. 1982. Use of iron milk medium for enumeration of *Clostridium perfrigens. J. Assoc. Off. Anal. Chem.* 65: 1129-1133.

Stedman's medical dictionary. 1982. 24th ed. Williams & Wilkins, Baltimore, MD.

Sumner, B.E.H. 1988. *Basic histochemistry.* John Wiley and Sons, New York, NY.

Swartz, R.C., W.A. DeBen, K.A. Serco, and J.O. Lamberson. 1982. Sediment toxicity and the distribution of amphipods in Commencement Bay, Washington, USA. *Mar. Poll. Bull.* 13(10):359-364.

Swartz, R.C., D.W. Schultz, G.R., Ditsworth, W.A. DeBen, and F.A. Cole. 1985. Sediment toxicity, contamination, and macrobenthic communities near a large sewage outfall. In *Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystems*, ed. T.P. Boyle, pp. 152-175. ASTM STP 865. American Society for Testing and Materials, Philadelphia, PA.

Taber's cyclopedic medical dictionary. 1985. F. A. Davis Company, Philadelphia, PA.

Taub, F.B. 1984. Measurement of pollution in standardized aquatic microcosms. In *Concepts in marine pollution measurements*, ed. H.H. White, pp. 159-192. Maryland Sea Grant College, College Park, MD.

Tetra Tech. 1985. *Bioaccumulation monitoring guidance: Recommended analytical detection limits.* Vol. 3. Tetra Tech, Inc., Bellevue, WA.

Tetra Tech. 1986. *Bioaccumulation monitoring guidance: Analytical methods for U.S. EPA priority pollutants and 301(h) pesticides in tissues from estuarine and marine organisms.* Vol. 4. Tetra Tech, Inc., Bellevue, WA.

Tetra Tech. 1987. *Bioaccumulation monitoring guidance: Strategies for sample replication and compositing.* Vol. 5. Tetra Tech, Inc.

Thomann, R.V., and J.A. Mueller. 1987. *Principles of surface water quality modeling and control.* Harper and Row Publ., New York, NY.

Thomas, P. 1990. Molecular and biochemical responses of fish to stressors and their potential use in environmental monitoring. *Proceedings American Fisheries Society Symposium* 8: 9-28, Washington, DC.

Tomascik, T., and F. Saunder. 1987. Effects of eutrophication on reef-building corals. II. Structure of scleractinian coral communities on fringing reefs, Barbados, West Indies. *Mar. Biol.* 94: 77-94.

Toole, C.L., R.A. Barnhart, and C.P. Onuf. 1987. *Habitat suitability index models: Juvenile English sole.* Biological reports of the U.S. Fish and Wildlife Service, Washington, DC.

Tsai, Chu-Fa. 1968. Effects of chlorinated sewage effluents on fishes in Upper Patuxent River, Maryland. *Chesapeake Sci.* 9: 83-93.

Turgeon, D.D., S.B. Bricker, and T.P. O'Connor. 1991. National status and trends program: Chemical and biological monitoring of U.S. coastal waters. In *Ecological indicators*, Vol. 1, ed. D.H. McKenzie and D.E. Hyatt. Elsevier Applied Science, Essex, England.

UNESCO. 1968. *Monographs on oceanographic methodology. 2. Zooplankton sampling.* United Nations Education, Scientific, and Cultural Organization, Paris, France.

UNESCO. 1973. *Monographs on oceanographic methodology. 3. A guide to the measurement of marine primary production under some special conditions.* United Nations Education, Scientific, and Cultural Organization, Paris, France.

UNESCO. 1988. *The acquisition, calibration, and analysis of CTD data.* UNESCO technical paper in marine science 54. United Nations Educational, Scientific, and Cultural Organization, France.

USEPA. 1978. *Microbiological methods for monitoring the environment.* EPA 600/8-78-017. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory.

USEPA. 1980. Ocean discharge criteria, final rule. *Fed. Reg.*, Vol. 45, No. 194, 65942-65954.

USEPA. 1982a. *Design of 301(h) monitoring programs for municipal wastewater discharges to marine waters.* EPA 430/9-82-010. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1982b. *Handbook for sampling and sample preservation of water and wastewater.* EPA 600/4-82-019. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

USEPA. 1982c. *Method for use of caged mussels to monitor for bioaccumulation and selected biological responses of toxic substances in municipal wastewater discharges to marine waters.* Draft. U.S. Environmental Protection Agency, Environmental Monitoring Support Laboratory, Cincinnati, OH.

USEPA. 1983a. *Methods for chemical analysis of water and wastes.* EPA 600/4-79-020. U.S. Environmental Protection Agency, Environmental Support Laboratory, Cincinnati, OH.

USEPA. 1983b. *Project summary: Experimental marine microcosm test protocol and support document.* EPA-600/S3-83-055. U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, RI.

USEPA. 1985a. *Bioaccumulation monitoring guidance: Selection of target species and review of available bioaccumulation data.* Vol. 2. EPA 403/9-86-006. Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1985b. *Interim guidance on quality assurance/quality control (QA/QC) for the estuarine field and laboratory methods.* U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1985c. *Methods for measuring the acute toxicity of effluents freshwater and marine organisms.* EPA 600/4-85-013. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

USEPA. 1985d. *Recommended biological indices for 301(h) monitoring programs.* EPA 430/9-86-002. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1985e. *Test methods for* Escherichia coli *and enterococci in water by the membrane filter procedure.* U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

USEPA. 1986-1988. *Quality criteria for water.* EPA 440/5-86-001. U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC.

USEPA. 1986-1991. *Recommended protocols for measuring selected environmental variables in Puget Sound.* Looseleaf. U.S. Environmental Protection Agency, Region 10, Puget Sound Estuary Program, Seattle, WA.

USEPA. 1986a. *Analytical methods for USEPA priority pollutants and 301(h) pesticides in estuarine and marine sediments.* Prepared for the Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1986b. *Bioaccumulation monitoring guidance: Analytical methods for USEPA priority pollutants and 301(h) pesticides in tissues from estuarine and marine organisms.* Vol. 4. EPA 503/6-90-002. Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1986c. *Proceedings and summary of the workshop on finfish as indicators of toxic contamination.* July 27-28, 1986, Arlie, VA. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1986d. *Quality criteria for water.* EPA 440/5-86-001. U.S. Environmental Protection Agency, Office of Water Regulations and Standards. Washington, DC.

USEPA. 1986e. *Test methods for evaluating solid wastes, physical/chemical methods.* SW-846, 3d ed. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1987a. *Bioaccumulation monitoring guidance: Strategies for sample replication and compositing.* Vol. 5. EPA 430-9-87-003. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1987b. *Guidance for conducting fish liver histopathology studies during 301(h) monitoring.* EPA 430/09-87-004. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1987c. *Quality assurance/quality control (QA/QC) for 301(h) monitoring program: Guidance on field and laboratory methods.* EPA 430/9-86-004. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1987d. *Technical support document for ODES statistical power analysis.* EPA 430/9-87-005. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1988a. *Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures.* Draft EPA research series report. EPA 600/3-88-034. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

USEPA. 1988b. *Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures.* Draft EPA research series report. EPA 600/3-88-035. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

USEPA. 1988c. *Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures.* Draft phase III toxicity series report. EPA/600/3-88-036. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

USEPA. 1988d. *ODES data brief: Use of numerical classification.* U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1988e. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms.* EPA 600/4-87-02. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.

USEPA. 1988f. *Water quality standards criteria summaries: A compilation of state/federal criteria - Bacterial.* U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC.

USEPA. 1989a. *Assessing human health risks from chemically contaminated fish and shellfish: A guidance manual.* U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1989b. *Biomonitoring for control of toxicity in effluent discharges to the marine environment.* EPA 625/8-89-015. U.S. Environmental Protection Agency, Office of Research and Development, Center for Environmental Research Information.

USEPA. 1989c. *Chesapeake Bay basin monitoring program atlas* (Vols. 1 and 2). U.S. Environmental Protection Agency, Chesapeake Bay Liaison Office, Annapolis, MD.

USEPA. 1989d. *Guidance manual: Bedded sediment bioaccumulation tests.* EPA/600/X-89/302. ERLN-N111. U.S. Environmental Protection Agency, Environmental Research Laboratory, Newport, OR.

USEPA. 1989e. *Rapid bioassessment protocols for use in streams and rivers.* EPA/444/4-89-001. Washington, DC

USEPA. 1990a. *Assessment and control of bioconcentratable contaminants in surface waters.* Draft report. U.S. Environmental Protection Agency, OWEP, Washington, DC.

USEPA. 1990b. *Compendium of methods for marine and estuarine environmental studies.* EPA 503/2-89/001. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

USEPA. 1990c. *Computerized Risk and Bioaccumulation System* (Version 1.0). ERLN-N137. U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, RI.

USEPA. 1990d. *Environmental Monitoring and Assessment Program: Ecological indicators.* EPA 600/3-90-060. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

USEPA. 1990e. *Experimental marine microcosm test protocol and support document.* Revised. U.S. Environmental Protection Agency, Environmental Research Laboratory, Ecosystems Effects Branch, Narragansett, RI.

USEPA. 1990f. *Statement of work for inorganics analysis: Multi-media, multi-concentration.* Document no. ILM01.0. U.S. Environmental Protection Agency, Contract Laboratory Program, Washington, DC.

USEPA. 1991a. *Biological criteria: Research and regulation.* EPA-440/5-91-005. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

USEPA. 1991b. *Biological criteria: Guide to technical literature.* EPA-440/5-91-004. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

USEPA. 1991c. *Technical support document for water quality-based toxics control.* EPA/505/2-90-001. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1992. *Sediment classification methods compendium.* Prepared for the U.S. Environmental Protection Agency, Office of Water Regulations and Standards.

USEPA/COE. 1977. U.S. Environmental Protection Agency and U.S. Army Corps of Engineers. *Ecological evaluation of proposed discharge of dredged material into ocean water.* Environmental Effects Laboratory, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

USFWS. 1980. *Habitat evaluation procedures (HEP) manual (102ESM).* U.S. Department of the Interior, Fish and Wildlife Service, Washington, DC.

Vogelbein, W.K., J.W. Fournie, P.A. van Veld, and R.J. Hugget. 1990. Hepatic neoplasms in the mummichog *Fundulus heterodotus* from a creosote-contaminated site. *Cancer Res.* 50:5978-5986.

Ward, R.C., and J.C. Loftis. 1986. Establishing statistical design criteria for water quality monitoring systems, review and synthesis. *Water Res. Bull.* 22(5): 759-767.

Warwick, R.M. 1985. A new method for detecting pollution effects on marine macrobenthic communities. *Mar. Biol.* 92: 557-562.

Warwick, R.M. 1986. The level of taxonomic discrimination required to detect pollution effects on marine benthic communities. *Mar. Poll. Bull.* 19: 259-268.

Washington State Department of Ecology. 1991. Sediment management standards. Washington Administrative Code (WAC) Chapter 173-204. Olympia, WA.

Weber, C.I. 1973. *Biological field and laboratory methods for measuring the quality of surface waters and effluents.* EMSL-Cincinnati. EPA 670/4-73/001.

Wedemeyer G., R. Gould, and W. Yasutake. 1983. Some potentials and limits of the leucocrit test as a fish health assessment method. *J. Fish Biol.* 23: 711.

Wedemeyer, G., and W. Yasutake. 1977. *Clinical methods for the assessment of the effects of environmental stress on fish health.* Technical paper 89. U.S. Fish and Wildlife Service, U.S. Department of the Interior, Washington, DC.

Weeks, B., R. Huggett, J. Warinner, and E. Mathews. 1990. Macrophage responses of estuarine fish as bioindicators of toxic contamination. In *Biomarkers of environmental contamination*, ed. J. McCarthy and L. Shugart. CRC Press, Boca Raton, FL.

Weinberg, S. 1981. A comparison of coral reef survey methods. *Bijdragen tot de Dierkunde* 51(2): 199-218.

Weis, J.S., P. Weis, and E.J. Zimmerer. 1990. Potential utility of fin regeneration in testing sublethal effects of wastes. In *Oceanic processes in marine pollution.* Vol. 6, Physical and chemical processes: Transport and transportation, ed. D. Baumgortner and I. Duedall. Krieger Pub. Co.

West, G. 1990. Methods of assessing ovarian development in fishes: A review. *Aust. J. Mar. Freshwater Res.* 41: 199-222.

Whipple, J.A., M. Jung, R.B. MacFarlane, and R. Fischer. 1984. *Histopathological manual for monitoring health of striped bass in relation to pollutant burdens.* NOAA Tech. Mem. TM-NMFS-SWFC-46. U.S. Department of Commerce, National Marine Fisheries Service.

White, M.W., and J.W. Porter. 1985. The establishment and monitoring of two permanent photograph transects in Looe Key and Key Largo National Marine Sanctuaries (Florida Keys). *Proc. 5th Int. Coral Reef Congr.* 6: 531-537.

Wiebe, P.H., and W.R. Holland. 1968. Plankton patchiness: Effects on repeated net tows. *Limno. Oceanogr.* 13: 315-321.

Wilson. 1986. *Techniques of water-resources investigations of the United States Geological Survey. Fluorometric procedures for dye tracing.* U.S. Department of the Interior, U.S. Geological Survey, Washington, DC.

Witman, J.D. 1985. Refuges, biological disturbance, and rocky subtidal community structure in New England. *Ecol. Mono.* 55: 421-445.

Word, J.Q. 1978. *The infaunal trophic index.* 1978 Annual Report, Southern California Coastal Water Research Project, pp. 19-39.

Wright, S.J. 1988. Outfall plume dilution in stratified fluids. *Proc. Int. Symp. Model-Prototype Correlation of Hydraul. Structures* 148.

Yevich, P.P., and C.A. Barszcz. 1980. Preparation of aquatic animals for histopathological examination. In *International mussel watch*, Appendix 6-13, pp. 212-220. U. S. National Academy of Sciences, Washington, DC.

Yevich, P.P., and C.A. Barszcz. 1983. Histopathology as a monitor for marine pollution: Results of histopathological examinations of the animals collected for the 1976 Mussel Watch Program. *Rapp. P. -v. Reun. Cons. Int. Explor. Mer* 182: 96-102.

APPENDIX A:
MONITORING METHODS REFERENCES

PHYSICAL CHARACTERISTICS

APHA. 1989. American Public Health Association, American Water Works Association, and Water Pollution Control Federation. *Standard methods for the examination of water and wastewater.* 17th ed. American Public Health Association, Washington, DC.

Appell, G.F., and T.B. Curtin. 1990. *Proceedings of the IEEE Fourth Working Conference on Current Measurement.* Institute of Electrical and Electronic Engineers, New York, NY.

Appell, G.F., and W.E. Woodward. 1986. *Proceedings of the IEEE Third Working Conference on Current Measurement.* Institute of Electrical and Electronic Engineers, New York, NY.

Brainard, E.C., and R.J. Lukens. 1975. *A comparison of the accuracies of various continuous recording current meters for offshore use.* Offshore technology conference, Paper no. OTC 2295.

Clausner, J.E., W.A. Birkemeier, and G.R. Clarke. 1986. *Field comparison of four nearshore survey systems.* Miscellaneous Paper CERC-86-6. U.S. Army Engineer Waterways Experimental Station, Vicksburg, MS.

Davis, R.E. 1988. Modeling eddy transport of passive tracers. *J. Mar. Res.* 45: 635.

Day, J.W., C.A.S. Hall, W.M. Kemp, and A. Yanez-Arancibia. 1989. *Estuarine ecology.* John Wiley and Sons, New York, NY.

Demmann, W.P., J.R. Proni, J.F. Craynock, and R. Fargen. In press. *Oceanic wastewater outfall plume characterization measured acoustically.*

Eugene, L.B. 1966. *Handbook of oceanographic tables.* Naval Oceanographic Office, Rep. SP-68.

Fofonoff, N.P., and R.C. Millard, Jr. 1983. *Algorithms for computation of fundamental properties of seawater.* Technical papers in marine science no. 44. United Nations Educational, Scientific, and Cultural Organization (UNESCO), Paris, France.

Grace, R.A. 1978. *Marine outfall systems planning, design, and construction.* Prentice Hall, Inc., Englewood Cliffs, NJ.

Hill K.D., T.M. Dauphinee, and D.J. Woods, 1986. Extension of the practical salinity scale 1978 to low salinities. *IEEE Journal of Oceanic Engineering* OE-11 (1, January 1986): 109-112.

Johns, W.E. 1988. Near-surface current measurements in the Gulf Stream using an upward-looking acoustic doppler current meter. *J. Atmos. Ocean. Technol.* 5:602.

Koh, R.C.Y. 1988. Shoreline impact from ocean waste discharges. *J. Hydraul. Div. Am. Soc. Civ. Eng.* 114:361.

Leighton, J.P., et al. 1988. Verification/Calibration of a thermal discharge model. *Proc. Int. Symp. Model-Prototype Correlation of Hydraul. Structures* 148.

McCullough, J.R. 1977. Problems in measuring currents near the ocean surface. *Proceedings of Oceans* '77, Marine Tech. Soc. and Inst. of Electrical and Electronics Engineering.

Pickard, G.L., and W.J. Emery. 1982. *Descriptive physical oceanography, An introduction.* 4th (SI) enlarged ed. Pergamon Press, Inc., New York, NY.

Pond, S., and G.L. Pickard 1983. *Introductory dynamic oceanography.* 3d ed. Pergamon Press, Inc., New York, NY.

Thomann, R.V., and J.A. Mueller. 1987. *Principles of surface water quality modeling and control.* Harper and Row Publ., New York, NY.

USEPA. 1982. *Design of 301(h) monitoring programs for municipal wastewater discharges to marine waters.* EPA 430/9-82-010. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1983. *Methods for chemical analysis of water and wastes.* EPA 600/4-79-020. U.S. Environmental Protection Agency, Environmental Support Laboratory, Cincinnati, OH.

USEPA. 1985. *Initial mixing characteristics of municipal ocean discharges.* Vol. I, Procedures and applications. EPA-600/3-85-073a. U.S. Environmental Protection Agency, Narragansett, RI.

USEPA. 1986. *Quality criteria for water.* EPA 440/5-86-001. U.S. Environmental Protection Agency, Office of Water Regulations and Standards. Washington, DC.

USEPA. 1987. *Quality assurance/quality control (QA/QC) for 301(h) monitoring program: Guidance on field and laboratory methods.* EPA 430/9-86-004. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

UNESCO. 1988. *The acquisition, calibration, and analysis of CTD data.* UNESCO technical paper in marine science 54. United Nations Educational, Scientific, and Cultural Organization, France.

Wallace, J.W., and J.W. Cox. 1976. *Design, fabrication and system integration of a satellite tracked, free-drifting ocean data buoy.* NASA Technical Memorandum X-72817. January.

Wilson et al. 1986. *Techniques of water-resources investigations of the United States Geological Survey. Fluorometric procedures for dye tracing.* U.S. Department of the Interior, U.S. Geological Survey, Washington, DC.

Wright, S.J., et al. 1988. Outfall plume dilution in stratified fluids. *Proc. Int. Symp. Model-Prototype Correlation of Hydraul. Structures* 148.

WATER CHEMISTRY

APHA. 1989. American Public Health Association, American Water Works Association, Water Control Pollution Federation. *Standard methods for the examination of water and wastewater.* 17th ed. American Public Health Association, Washington, DC.

Armstrong, J.W., and A.E. Copping. 1989. Comparing the Regional Puget Sound Marine Monitoring with the NOAA National Status and Trends Program. *Coastal Zone Proceedings* 3: 2421-2435.

Becker, D.S., and J.W. Armstrong. 1988. Development of regionally standardized protocols for marine environmental studies. *Mar. Poll. Bull.* 19(7): 310-313.

D'Elia, C.F., et al. 1987. *Nitrogen and phosphorus determinations in estuarine waters: A comparison of methods used in Chesapeake Bay monitoring.* Final report to the Chesapeake Bay Program, U.S. Environmental Protection Agency, Region III. U.S. Government Printing Office, Washington, DC.

D'Elia, C.F., N.L. Kaumeyer, C.W. Keefe, K.V. Wood, and C.F. Zimmerman. 1988. *Nutrient analytical services laboratory standard operating procedures.* Chesapeake Biological Laboratory, University of Maryland.

D'Elia, C.F., J.G. Sanders, and D.G. Capone. 1989. Analytical chemistry for environmental sciences: A question of confidence. *Environ. Sci. Technol.* 23(7):768-774.

D'Elia, C.F., P.A. Steudler, and N. Corwin. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnol. Oceanogr.* 22: 760-764.

Ferraro, S.P., F.A. Cole, W.A. DeBen, and R.C. Swartz. 1989. Power-cost efficiency of eight macrobenthic sampling schemes in Puget Sound, WA, USA. *Can. J. Fish. Aquat. Sci.* 46: 2157-2165.

Gilliom, R.J., and D.R. Helsel. 1986. Estimation of distributional parameters for censored trace level water quality data. 1, Estimation Techniques. *Water Res. Res.* 22(2): 135-146.

Glibert, P.M., and T.C. Loder. 1977. *Automated analysis of nutrient seawater: Manual of techniques.* Woods Hole Oceanographic Institute Technical Report No. WH01-77-47.

Goldberg, E.D., V.T. Bowen, G.H. Farrington, J.H. Martin, P.L. Parker, R.W. Risebrough, W. Robertson, E. Schneider and E. Gamble. 1978. The mussel watch. *Environ. Conserv.* 5:101-125.

Hirsch, R.M. 1988. Statistical methods and sampling design for estimating step trends in surface-water quality. *Water Res. Bull.* 24(3): 493-503.

Ladd, J.M., S.P. Hayes, M. Martin, M.D. Stephenson, S.L. Coale, J. Linfield, and M. Brown. 1984. *California state mussel watch: 1981-1983. Trace metals and synthetic organic compounds in mussels from California's coast, bays and estuaries.* Biennial report. Water Quality Monitoring Report No. 83-6TS. Sacramento, CA.

Porter, P.S., R.C. Ward, and H.F. Bell. 1988. The detection limit. *Env. Sci. Tech.* 22: 856-861.

Salley, B.A., J.G. Bradshaw, and B.J. Neilson. 1986. *Results of comparative studies of preservation techniques for nutrient analysis on water samples.* Virginia Institute of Marine Science Report to the Chesapeake Bay Liaison Office. September.

Sokal, R.R. and F.J. Rohlf. 1981. *Biometry.* W.H. Freeman and Co., San Francisco, CA.

Strickland, J.D.H., and T.R. Parsons. 1968. *A practical handbook of seawater analysis.* Fisheries Research Board of Canada, Ottawa, Canada.

Tetra Tech. 1990. *National Estuary Program monitoring guidance document.* Draft report. Prepared for U.S. Environmental Protection Agency by Tetra Tech, Inc., Lafayette, CA.

USEPA. 1979. *Handbook for analytical quality control in water and wastewater laboratories.* EPA 600/4-79-019. Environmental Monitoring and Support Laboratory, Cincinnati, OH.

USEPA. 1982. *Methods for organic chemical analysis of municipal and industrial wastewater.* EPA 600/4-82-057. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

USEPA. 1983. *Methods for chemical analysis of water and wastes.* 2d ed. EPA 600/4-79-020. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

USEPA. 1986. *Test methods for evaluating solid waste.* EPA Publication SW-846, 3d ed. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC.

USEPA. 1986-1991. *Recommended protocols for measuring selected environmental variables in Puget Sound.* Looseleaf. U.S. Environmental Protection Agency, Region 10, Puget Sound Estuary Program, Seattle, WA.

USEPA. 1987. *Quality assurance/quality control (QA/QC) for 301(h) monitoring programs: Guidance on field and laboratory methods.* EPA 430/9-86-004. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1990. *Compendium of methods for marine and estuarine environmental studies.* EPA 503/2-89/001. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Valderama, J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar. Chem.* 10: 109-122.

Ward, R.C., and J.C. Loftis. 1986. Establishing statistical design criteria for water quality monitoring systems, review and synthesis. *Water Res. Bull.* 22(5): 759-767.

SEDIMENT CHEMISTRY

APHA. 1989. American Public Health Association, American Water Works Association, Water Pollution Control Federation. *Standard methods for the examination of water and wastewater.* 17th ed. American Public Health Association, Washington, DC.

ASTM. 1990. Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. ASTM Guide E1367-90. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1991. Standard guide for collection, storage, characterization, and manipulation of sediments for toxicological testing. ASTM Designation E1391-90. In *Annual book of ASTM standards.* American Society for Testing and Materials, Philadelphia, PA.

DiToro, D.M., J.D. Mahony, D.J. Hansen, K.J. Scott, A.R. Carlson, and G.T. Ankley. In press. *Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments.*

DiToro, D.M., J.D. Mahony, D.J. Hansen, K.J. Scott, M.B. Hicks, S.M. Mayr, and M.S. Redmond. In press. *Toxicity of cadmium in sediments: The role of acid volatile sulfide.*

EA and Battelle. 1990. EA Engineering, Science, and Technology, Inc. and Battelle Ocean Sciences. *Evaluation of dredged material proposed for ocean disposal (testing manual).* Draft report to U.S. Envrionmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

Eganhouse, R.P. 1990. Sources and magnitude of error associated with PCM measurements. In *Southern California Coastal Water Research Project annual report 1989-1990*, ed. J.N. Cross and D.M. Wiley. Southern California Coastal Water Research Project, Long Beach, CA.

Ferraro, S.P., H. Lee, R.J. Ozretich, and D.T. Specht. 1990. Predicting bioaccumulation potential: A test of a fugacity-based model. *Arch. Environ. Contam. Tocicol.* 19:386-394.

Fredette, T.J., D.A. Nelson, T. Miller-Way, J.A. Adair, V.A. Sotler, J.E. Clausner, E.B. Hands, and F.J. Anders. 1989. *Selected tools and techniques for physical and biological monitoring of aquatic dredged material disposal sites.* Final report. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Hiatt, M.H. 1981. Analysis of fish and sediment for volatile priority pollutants. *Anal. Chem.* 53: 1541-1543.

Karickhoff, S.W., D.S. Brown, and T.A. Scott. 1979. Sorption of hydrophobic pollutants on natural sediments. *Wat. Res.* 13: 241-248.

Knezovich, J.P., and F.L. Harrison. 1987. A new method for determining the concentration of volatile organic compounds in sediment interstitial water. *Bull. Environ. Contam. Toxicol.* 38: 837-940.

Lake, J.L., N.I. Rubenstein, and S. Parvigano. 1987. Predicting bioaccumulation: Development of a partitioning model for use as a screen tool in regulating ocean disposal of wastes. In *Fate and effects of sediment-bound chemicals in aquatic systems,* ed. K.L. Dickson, A.W. Maki, and W.A. Brungo. Sixth Pellston Workshop, Florissano, CO.

Landrum, P.F., and J.A. Robbins. In press. Bioavailability of sediment-associated contaminants to benthic invertebrates. In *Sediments: Chemistry and toxicity of in-place pollutants,* ed. J.P. Giesy, R. Baudo, and H. Muntau. Lewis Publishers.

McFarland, V.A., J.U. Clarke, and A.B. Gibson. 1986. *Changing concepts and improved methods for evaluating the importance of PCBs as dredged sediment contaminants.* Miscellaneous Paper D-86-5. Department of the Army, Corps of Engineers, Waterways Experiment Station, Vicksburg, MS.

McIntyre, A.D., J.M. Elliot, and D.V. Ellis. 1984. Introduction: Design of sampling programs. IBP handbook no. 16. In *Methods for the study of marine benthos*, ed. N.A. Holme and A.D. McIntyre. Blackwell Scientific Publications, Oxford.

Plumb, R.H., Jr. 1981. *Procedure for handling and chemical analysis of sediment and water samples.* Technical report EPA/CE-81-1. Prepared for the U.S. Environmental Protection Agency/Corps of Engineers Technical Committee on Criteria for Dredged and Fill Material, by Great Lakes Laboratory, State University College at Buffalo, Buffalo, NY. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Swartz, R.C., W.A. DeBen, K.A. Serco, and J.O. Lamberson. 1982. Sediment toxicity and the distribution of amphipods in Commencement Bay, Washington, USA. *Mar. Poll. Bull.* 13(10):359-364.

Swartz, R.C., D.W. Schultz, G.R., Ditsworth, W.A. DeBen, and F.A. Cole. 1985. Sediment toxicity, contamination, and macrobenthic communities near a large sewage outfall. In *Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystems*, ed. T.P. Boyle, pp. 152-175. ASTM STP 865. American Society for Testing and Materials, Philadelphia, PA.

USEPA. 1986. *Analytical methods for USEPA priority pollutants and 301(h) pesticides in estuarine and marine sediments.* Prepared for the Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1986. *Test methods for evaluating solid wastes, physical/chemical methods.* SW-846, 3d ed. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1986-1991. *Recommended protocols for measuring selected environmental variables in Puget Sound.* Looseleaf. U.S. Environmental Protection Agency, Region 10, Puget Sound Estuary Program, Seattle, WA.

USEPA. 1987. *Bioaccumulation monitoring guidance: Strategies for sample replication and compositing.* Vol. 5. EPA 430/9-87-003. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1987. *Quality assurance/quality control (QA/QC) for 301(h) monitoring programs*: Guidance on field and laboratory methods. EPA 430/9-86-004. Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1989. *Sediment classification methods compendium.* Prepared for the U.S. Environmental Protection Agency, Office of Water Regulations and Standards.

USEPA. 1990. *Statement of work for inorganics analysis: Multi-media, multi-concentration.* Document no. ILM01.0. U.S. Environmental Protection Agency, Contract Laboratory Program, Washington, DC.

USEPA/COE. 1977. U.S. Environmental Protection Agency and U.S. Army Corps of Engineers. *Ecological evaluation of proposed discharge of dredged material into ocean water.* Environmental Effects Laboratory, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Washington State Department of Ecology. 1991. Sediment management standards. Washington Administrative Code (WAC) Chapter 173-204. Olympia, WA.

SEDIMENT GRAIN SIZE

ASTM. 1991. Standard guide for collection, storage, characterization, and manipulation of sediments for toxicological testing. ASTM designation E1391-90. In *Annual book of ASTM standards.* American Society for Testing and Materials, Philadelphia, PA.

Buller, A.T., and J. McManus. 1979. Sediment sampling and analysis. In *Estuarine hydrography and sedimentation*, ed. K.R. Dyer. Cambridge University Press, Cambridge.

Folk, R.L. 1980. *Petrology of sedimentary rocks.* Herpmill Publishing Co., Austin, TX.

Fredette, T.J., D.A. Nelson, T. Miller-Way, J.A. Adair, V.A. Sotler, J.E. Clausner, E.B. Hands, and F.J. Anders. 1989. *Selected tools and techniques for physical and biological monitoring of aquatic dredged material disposal sites.* Final report. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

McIntyre, A.D., J.M. Elliot, and D.V. Ellis. 1984. Introduction: Design of sampling programs. IBP Handbook No. 16. In *Methods for the study of marine benthos*, ed. N.A. Holme and A.D. McIntyre, pp. 1-26. Blackwell Scientific Publications, Oxford.

Plumb, R.H., Jr. 1981. *Procedure for handling and chemical analysis of sediment and water samples.* Technical report EPA/CE-81-1. Prepared for the U.S. Environmental Protection Agency/Corps of Engineers Technical Committee on Criteria for Dredged and Fill Material, by Great Lakes Laboratory, State University College at Buffalo, Buffalo, NY. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Shepard, F.P. 1954. Nomenclature based on sand-silt-clay ratios. *J. Sed. Petrol.* 24(3):151-158.

USEPA. 1986-1991. *Recommended protocols for measuring selected environmental variables in Puget Sound.* Looseleaf. U.S. Environmental Protection Agency, Region 10, Puget Sound Estuary Program, Seattle, WA.

USEPA. 1987. *Technical support document for ODES statistical power analysis.* EPA 430/9-87-005. Office of Marine and Estuarine Protection, Washington, DC.

BENTHIC COMMUNITY STRUCTURE

Amjad, S., and J.S. Gray. 1983. Use of the nematode/copepod ratio as an index of organic pollution. *Mar. Poll. Bull.* 14: 178-181.

Anderberg, M.R. 1973. *Cluster analysis for applications.* Academic Press, New York, NY.

ASTM. 1991. Standard guide for collection, storage, characterization, and manipulation of sediments for toxicological testing. ASTM desgination E1391-90. In *Annual book of ASTM standards.* American Society for Testing and Materials, Philadelphia, PA.

Avent, R.M., M.E. King, and R.H. Gore. 1977. Topographic and faunal studies of shelf edge prominences off the central eastern Florida coast USA. *Int. Rev. Gesamten Hydrobiol. 62(2): 185-208.*

Bazzaz, F.A. 1983. Characteristics of populations in relation to disturbance in natural and man-modified ecosystems. In *Disturbance and ecosystems*, ed. H. A. Mooney and M. Godron. Springer-Verlag, Berlin.

Becker, D.S., and J.W. Armstrong. 1988. Development of regionally standardized protocols for marine environmental studies. *Mar. Pollut. Bull.* 19(7):310-313.

Bernstein, B.B., and R.W. Smith. 1986. Community approaches to monitoring. *IEEE Conference Proceedings*, Oceans '86, pp. 934-939.

Beukema, J.J. 1988. An evaluation of the ABC method (abundance-biomass comparison) as applied to macrozoobenthic communities living on tidal flats in the Dutch Wadden Sea. *Mar. Biol.* 99: 425-433.

Bilyard, G.R. 1987. The value of benthic infauna in marine pollution monitoring studies. *Mar. Poll. Bull.* 18: 581-585.

Boesch, D.F. 1977. *Application of numerical classification in ecological investigations of water pollution.* EPA 600/3-77-033. U.S. Environmental Protection Agency, Office of Research and Development, Corvallis, OR.

Bohnsack, J.A. 1979. Photographic quantitative sampling of hard-bottom benthic communities. *Bull. Mar. Sci.* 29:242-252.

Brown, B.E. 1988. Assessing environmental impacts on coral reefs. *Proc. 6th Int. Coral Reef Symp.* 1:71-80.

Clifford, H.T., and W. Stephenson. 1975. *An introduction to numerical classification.* Academic Press, New York, NY.

Connell, J.H. 1978. Diversity in tropical rain forests and coral reefs. *Science* 199: 1302-1309.

Connell, J.H., and M.J. Keough. 1985. Disturbance and patch dynamics of subtidal marine animals on hard substrata. In *The ecology of natural disturbance and patch dynamics*, ed. S.T.A. Pickett and P.S. White. Academic Press, New York, NY.

Darcy, G.H. and E.J. Gutherz. 1984. Abundance of demersal fishes on the west Florida shelf, January 1978. *Bull. Mar. Sci.* 34:81-105.

Dayton, P.K. 1985. The structure and regulation of some South American kelp communities. *Ecol. Monagr.* 55(4):447-468.

Dodge, R.E., A. Logan, and A. Antonius. 1982. Quantitative reef assessment studies in Bermuda: A comparison of methods and preliminary results. *Bull. Mar. Sci.* 32(3):745-760.

Downing, J.A. 1979. Aggregation, transformation, and the design of benthos sampling programs. *J. Fish Res. Board Cer.* 36:1454-1463.

Dustan, P., and J.C. Halas. 1987. Changes in the reef-coral community of Carysfort Reef, Key Largo, Florida: 1974 to 1982. *Coral Reefs* 6(2):91-106.

Eleftheriou, A., and N.A. Holme. 1984. Macrofauna techniques. In *Methods for the study of marine benthos*, ed. N.A. Holmes and A.D. McIntyre, pp. 66-98. Blackwell Scientific Publications, Oxford.

Elliot, J.M. 1971. *Some methods for the statistical analysis of samples of benthic invertebrates.* Scientific Publication No. 25, Freshwater Biological Assn., Ferry House, UK.

Ellis, D. 1985. Taxonomic sufficiency in pollution assessment. *Mar. Poll. Bull.* 16: 459.

Elmgren, R., S. Hansson, U. Larsson, B. Sundelin, and P.D. Boehm. 1983. The "Tsesis" oil spill: Acute and long-term impact on the benthos. *Mar. Poll. Bull.* 15: 249-253.

Ferraro, S.P., F.A. Cole, W.A. DeBen, and R.C. Swartz. 1989. Power-cost efficiency of eight macrobenthic sampling schemes in Puget Sound, Washington, USA. *Can. J. Fish. Aquat. Sci.* 46: 2157-2165.

Fleishack, P.C., A.J. DeFreitas, and R.B. Jackson. 1985. Two apparatuses for sampling benthic fauna in surf zones. *Est. Cstl. Shelf Sci.* 21:287-293.

Fredette, T.J., D.A. Nelson, T. Miller-Way, J.A. Adair, V.A. Sotler, J.E. Clausner, E.B. Hands, and F.J. Anders. 1989. *Selected tools and techniques for physical and biological monitoring of aquatic dredged material disposal sites.* Final report. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Gamble, J.C. 1984. Diving. In *Methods for the study of marine benthos*, ed. N.A. Holmes and A.D. McIntyre, pp. 66-68. Blackwell Scientific Publications, Oxford.

Gauch, H.G., Jr. 1982. *Multivariate analysis* in community ecology. Cambridge University Press, New York, NY.

Gray, J.S., and F.B. Mirza. 1979. A possible method for the detection of pollution induced disturbance on marine benthic communities. *Mar. Poll. Bull.* 10: 142-146.

Green, R. 1979. *Sampling design and statistical methods for environmental biologists.* John Wiley and Sons, New York, NY.

Grigg, R.W., and S.J. Dollar. 1990. Natural and anthropogenic disturbance on coral reefs. In *Coral reefs*, ed. Z. Dubinsky, pp. 439-452. Ecosystems of the world 25. Elsevier, New York, NY.

Grizzle, R.E. 1984. Pollution indicator species of macrobenthos in a coastal lagoon. *Mar. Ecol. Prog. Ser.* 18: 191-200.

Hartley, J.P. 1982. Methods for monitoring offshore macrobenthos. *Mar. Poll. Bull.* 13:150-154.

Holme, N.A. 1984. Photography and television. IBP handbook no. 16. In *Methods for the study of marine benthos*, ed. N. A. Holmes and A. D. McIntyre, pp. 66-98. Blackwell Scientific Publications, Oxford.

Holme, N.A., and A.D. McIntyre, eds. 1984. *Methods for the study of marine benthos.* Blackwell Scientific Publications, Oxford.

Hurlbert, S.H. 1971. The nonconcept of species diversity: A critique and alternative parameters. *Ecology* 52: 577-586.

Jackson, J.B.C., J.D. Cubit, B.D. Keller, V. Batista, K. Burns, H.M. Caffey, R.L. Caldwell, S.D. Garrity, C.D. Getter, C. Gonzales, H.M. Guzman, K.W. Daufman, A.H. Knapp, S.C. Levings, M.J. Marshall, R. Steger, R.C. Thompson, and E. Weil. 1989. Ecological effects of a major oil spill on Panamanian coastal marine communities. *Science* 243: 37-44.

Lambshead, P.J. 1984. The nematode/copepod ratio. Some anomalous results from the Firth of Clyde. *Mar. Poll. Bull.* 15: 256-259.

Lambshead, P.J., and H.M. Platt. 1985. Structural patterns of marine benthic assemblages and their relationship with empirical statistical models. *Proc. 19th Eur. Mar. Biol. Symp.*, pp. 371-380.

Livingston, R.J., R.S. Lloyd, and M.S. Zimmerman. 1976. Determination of sampling strategy for benthic macrophytes in polluted and unpolluted coastal areas. *Bull. Mar. Sci.* 26:569-575.

Loya, Y. 1978. Plotless and transect methods. In *Coral reefs: Research methods.* ed. D. R. Stoddart and R. E. Johannes pp. 197-217. UNESCO, Monographs on Oceanographic Methodology, Paris.

Lunz, J.D., and D.R. Kendall. 1982. Benthic resource analysis technique, a method for quantifying the effects of benthic community changes on fish resources. In *Conference proceedings on marine pollution*, Oceans 1982, pp. 1021-1027. National Oceanic and Atmospheric Administration, Office of Marine Pollution Assessment, Rockville, MD.

McIntyre, A.D., J.M. Elliot, and D.V. Ellis. 1984. Introduction: Design of sampling programs. IBP handbook no. 16. In *Methods for the study of marine benthos*, ed. N.A. Holme and A.D. McIntyre, pp. 1-26. Blackwell Scientific Publications, Oxford.

Moore, E.J. 1978. Underwater photogrammetry. *Prog. in Und. Sci.* 3:101-110.

NRC. 1990. *Managing troubled waters: The role of marine environmental monitoring.* National Academy Press, Washington, DC.

Ohlhorst, S.L., W.D. Liddell, R.J. Taylor, and J.M. Taylor. 1988. Evaluation of reef census techniques. *Proc. 6th Int. Coral Reef Symp.* 2:319-324.

Palmer, M.A. 1988. Epibenthic predators and marine meiofauna: separating predation, disturbance, and hydrodynamic effects. *Ecology* 69:1251-1259.

Pearson, T.H., and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceangr. Mar. Biol. Ann. Rev.* 16: 229-311.

Phillips, N.W., D.A. Gettleson, and K.D. Spring. 1990. Benthic biological studies of the southwest Florida shelf. *Amer. Zool.* 30(1):65-75.

Pielou, E.C. 1984. *The interpretation of ecological data.* John Wiley and Sons, New York, NY.

Porter, J.W. 1972. Patterns of species diversity in Caribbean reef corals. *Ecology* 53:745-748.

Rabalais, N.N. 1990. Biological communities of the south Texas continental shelf. *Amer. Zool.* 30(1):77-87.

Raffaelli, D. 1987. The behavior of the nematode/copepod ratio in organic pollution studies. *Mar. Environ. Res.* 23: 135-152.

Raffaelli, D., and C.F. Mason. 1981. Pollution monitoring with meiofauna, using the ratio of nematodes to copepods. *Mar. Poll. Bull.* 12: 158-163.

Rees, H.L. 1984. A note on mesh selection and sampling efficiency in benthic studies. *Mar. Pollut. Bull.* 15:225-229.

Reish, D.J. 1959. A discussion of the importance of screen size in washing quantitative marine bottom samples. *Ecology* 40:307-309.

Reish, D.J. 1986. Benthic invertebrates as indicators of marine pollution: 35 years of study. *IEEE Conference Proceedings*, Oceans '86, pp. 885-888.

Rhoads, D.C., and J.D. Germano. 1982. Interpreting long-term changes in benthic community structure: A new protocol. *Hydrobiologia* 142: 291-308.

Rhoads, D.C., and J.D. Germano. 1986. Characterization of organism-sediment relations using sediment profile imaging: An efficient method of remote ecological monitoring of the sea floor (REMOTS system). *Mar. Ecol. Prog.* Ser. 8: 115-128.

Rhoads, D.C., and D.K. Young. 1970. The influence of deposit-feeding organisms on sediment stability and community trophic structure. *J. Mar. Res.* 28: 150-178.

Rogers, C.S. 1988. Recommendations for long-term assessment of coral reefs: U.S. National Park Service initiates regional program. *Proc. 6th Int. Coral Reef Symp.* 1:399-403.

Rogers, C.S., M. Gilnack, and H. C. Fitz III. 1983. Monitoring of coral reefs with linear transects: a study of storm damage. *J. Exp. Mar. Biol. Ecol.* 49:179-187.

Rogers, C.S., and E. Zullo. 1986. *Initiation of a long-term monitoring program for coral reefs in the Virgin Islands National Park.* Biosphere Reserve research report no. 17. Virgin Islands Resource Management Cooperative, U. S. National Park Service.

Romesburg, H.C. 1984. *Cluster analysis for researchers.* Lifetime Learning Publications, Belmont, CA.

Rygg, B. 1985. Distribution of species along pollution-induced diversity gradients in benthic communities in Norwegian fjords. *Mar. Poll. Bull.* 12: 469-474.

SCCWRP. 1988. *Recovery of Santa Monica Bay after termination of sludge discharge.* 1988-1989 Annual Report, Southern California Coastal Water Research Project, pp. 46-53.

Self, S.G., and R.H. Mauritsen. 1988. Power/sample size calculations for generalized linear models. *Biometrics* 44: 79-86.

Sheills, G.M., and K.J. Anderson. 1985. Pollution monitoring using the nematode/copepod ratio, a practical application. *Mar. Poll. Bull.* 16: 62-68.

Sneath, P.H.A. and R.R. Sokal. 1973. *Numerical taxonomy: The principles and practices of numerical classification.* Freeman, San Francisco, CA.

Swartz, R.C., D.W. Schultz, G.R. Ditsworth, W.A. DeBen, and F.A. Cole. 1985. Sediment toxicity, contamination, and macrobenthic communities near a large sewage outfall. In *Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystems*, ed. T.T. Boyle, pp. 152-175. American Society for Testing and Materials (ASTM), Philadelphia, PA.

Tomascik, T., and F. Sander. 1987. Effects of eutrophication on reef-building corals. II. Structure of scleractinian coral communities on fringing reefs, Barbados, West Indies. *Mar. Biol.* 94:77-94.

USEPA. 1985. *Recommended biological indices for 301(h) monitoring programs.* EPA 430/9-86-002. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1986-1991. *Recommended protocols for measuring selected environmental variables in Puget Sound.* Looseleaf. U.S. Environmental Protection Agency, Region 10, Puget Sound Estuary Program, Seattle, WA.

USEPA. 1987. *Technical support document for ODES statistical power analysis.* EPA 430/9-87-005. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1988. *ODES data brief: Use of numerical classification.* U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC..

USEPA. 1989. *Sediment classification methods compendium.* U.S. Environmental Agency, Office of Water Regulations and Standards, Washington, DC.

Warwick, R.M. 1985. A new method for detecting pollution effects on marine macrobenthic communities. *Mar. Biol.* 92:557-562.

Warwick, R.M. 1986. The level of taxonomic discrimination required to detect pollution effects on marine benthic communities. *Mar. Poll. Bull.* 19: 259-268.

Weinberg, S. 1981. A comparison of coral reef survey methods. *Bijdragen tot de Dierkunde* 51(2):199-218.

White, M.W., and J.W. Porter. 1985. The establishment and monitoring of two permanent photograph transects in Looe Key and Key Largo National Marine Sanctuaries (Florida Keys). *Proc. 5th Int. Coral Reef Congr.* 6:531-537.

Witman, J.D. 1985. Refuges, biological disturbance, and rocky subtidal community structure in New England. *Ecol. Mono.* 55:421-445.

Word, J.Q. 1978. *The infaunal trophic index.* 1978 Annual Report, Southern California Coastal Water Research Project, pp. 19-39.

Word, J.Q., B.L. Myers, and A.J. Mearns. 1977. *Animals that are indicators of marine pollution.* 1977 Annual Report, Southern California Coastal Water Research Project, pp. 199-207.

FISH AND SHELLFISH PATHOBIOLOGY

Adams, S.M., ed. 1990. *Biological indicators of stress in fish.* American Fisheries Society Special Symposium No. 8.

Adams, S.M., L.R. Shugart, and G.R. Southworth. 1990. Application of bioindicators in assessing the health of fish populations experiencing contaminant stress. In *Biomarkers of environmental contamination,* ed. J. McCarthy and L. Shugart. CRC Press, Boca Raton, FL.

Anderson, D. 1990. Immunological indicators: Effects of environmental stress on immune protection and disease outbreaks. *Proceedings American Fisheries Society Symposium* 8: 38-50, Washington, DC.

Anderson, D. 1990. Passive hemolytic plaque assay for detecting antibody-producing cells in fish. In *Techniques in fish immunology,* ed. J. Stolen, J. Fletcher, D. Anderson, B. Roberson, and W. van Muiswinkel. SOS Publications, Fair Haven, NJ.

Anderson, D., B. Roberson, and O. Dixon. 1979. Cellular immune response in Rainbow Trout *Salmo Gairdneri,* Richardson to *Yersinia Ruckeri* O-antigen monitored by the passive haemolytic plaque assay test. *J. Fish Dis.* 2: 169-178.

Anderson, D., O. Dixon, and E. Lizzio. 1986. Immunization and culture of Rainbow Trout organ sections *in vitro. Vet. Immunol. Immun.* 12: 203-211.

Anderson, D., O. Dixon, and W. van Muiswinkel. 1990. Reduction in the numbers of antibody-producing cells in rainbow trout, *Oncorhynchus mykiss,* exposed to sublethal doses of phenol before bath immunization. In *Aquatic toxicology,* ed. J. Nriagu. A Wiley-Interscience Publication, John Wiley and Sons, New York.

Anderson, R.S. 1987. Immunocompetence in invertebrates. In *Pollutant studies in marine animals,* ed. C. S. Giam and L. E. Ray. CRC Press, Boca Raton, FL.

Anderson, R.S. 1990. Effects of pollutant exposure on bactericidal activity of *Mercenaria mercenaria* hemolymph. In *Biological markers of environmental contaminants,* ed. J.F. McCarthy and L.R. Shugart. American Chemical Society, Los Angeles, CA.

Blaxhall, P., and K. Daisley. 1973. Routine haematological methods for use with fish blood. *J. Fish Biol.* 5: 771.

Bouck, G.R. 1984. Physiological responses of fish: Problems and progress toward use in environmental monitoring. In *Contaminant effects on fisheries*, ed. V. W. Cairns, P. V. Hodson, and J. O. Nriagu. John Wiley and Sons, New York, NY.

Brown, D.A., C.A. Bowden, K. Chatel, and T.R. Parsons. 1977. The wildlife community of Iona Island Jetty, Vancouver, BC, and heavy metal pollution effects. *Environ. Conserv.* 4: 213-216.

Brusick, D. 1980. *Principles of genetic toxicology.* Plenum Press, New York, NY.

Buckley, L.J., T.A. Halavik, G.C. Lawrence, S.J. Hamilton, and P. Yevich. 1985. Comparative swimming stamina, biochemical composition, backbone mechanical properties, and histopathology of juvenile striped bass from rivers and hatcheries of the eastern United States. *Trans. Amer. Fish. Soc.* 114:114-124.

Cailliet, G.M., M.S. Love, and A.W. Ebeling. 1986. *Fishes: A field and laboratory manual on their structure, identification, and natural history.* Wadsworth Publishing Company, Belmont, CA.

Couch, J.A. 1978. Diseases, parasites, and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic coasts of North America. *Fish. Bull.* 76:1-44.

Couch, J.A., and J.C. Harshbarger. 1985. Effects of carcinogenic agents on aquatic animals: An environmental and experimental overview. *Environ. Carcinogenesis Revs.* 3(1):63-105.

Cross, J., and J.E. Hose. 1988. Evidence for impaired reproduction in white croaker (*Genyonemus lineatus*) from contaminated areas off southern California. *Mar. Environ. Res.* 24: 185-188.

Dawe, C.J., J.C. Harshbarger, R. Wellings, and J.D. Strandberg. In press. *The pathobiology of spontaneous and induced neoplasms in fishes: Comparative characterization, nomenclature, and literature.* Academic Press, New York, NY.

Dixon. 1982. *Mar. Biolog. Let.* 3:155-161.

Dorigan, J.V., and F.L. Harrison. 1987. *Physiological responses of marine organisms to environmental stresses.* U. S. Department of Energy, Washington, DC.

Ellis, A. 1977. The leucocytes of fish: A review. *J. Fish Biol.* 11:453.

Elskus, A., and J. Stegeman. 1989. Induced cytochrome P-450 in *Fundulus heteroclitus* associated with environmental contamination by polychlorinated biphenyls and polynuclear aromatic hydrocarbons. *Mar. Environ. Res.* 27: 31-50.

Engel, D. 1987. Metal regulation and molting in the blue crab, *Callinectes Sapidus*: Copper, zinc and metallothionein. *Biol. Bull.* 172: 69-82.

Engel, D., and G. Roesijadi. 1987. Metallothioneins: A monitoring tool. In *Pollution physiology of estuarine organisms*, ed. W. Vernberg, A. Calabrese, F. Thurberg, and F. J. Vernberg, pp. 421-438. University of South Carolina Press.

Engel, D. 1988. The effect of biological variability on monitoring strategies: Metallothioneins as an example. *Water Res. Bull.* 24(5): 981-987.

Ferguson, H.W. 1989. *Systemic pathology of fish: A text and atlas of comparative tissue responses in diseases of teleosts.* Iowa State University Press, Ames, IA.

Fisher, W.S. 1988. *Disease processes in marine bivalve molluscs.* American Fisheries Society Special Publication 18.

Gardner, G.R., P.P. Yevich, J.C. Harshbarger, and A.R. Malcolm. 1991. Carcinogenicity of Black Rock Harbor sediment to the eastern oyster and trophic transfer of Black Rock Harbor carcinogens from the blue mussel to the winter flounder. *Environ. Health Perspect.* 90:53-66.

Garvey, J. 1990. Metallothionein: A potential biomarker of exposure to environmental toxins. In *Biomarkers of environmental contamination*, ed. J. McCarthy and L. Shugart. CRC Press, Boca Raton, FL.

Giam, C.S., and L.E. Ray. 1987. *Pollutant studies in marine animals.* CRC Press, Boca Raton, FL.

Haasch, M., P. Wejksnora, J. Stegeman, and J. Lech. 1989. Cloned rainbow trout liver P_1450 complementary DNA as a potential environmental monitor. *Toxicol. Appl. Pharm.* 98: 362-368.

Hargis, W., M. Roberts, and D. Zwerner. 1984. Effects of contaminated sediments and sediment-exposed effluent water on estuarine fish: Acute toxicity. *Mar. Environ. Res.* 14: 337-354.

Hargis. W., and D. Zwerner. 1988. Effects of certain contaminants on eyes of several estuarine fishes. *Mar. Environ. Res.* 24: 265-270.

Harrington, F.W., and J.O. Corliss. 1991. *Microscopic anatomy of invertebrates.* Vol. 1-15 (some still in press). Wiley-Liss, New York, NY.

Haux, C., and L. Forlin. 1988. *Biochemical methods for detecting effects of contaminants on fish.*

Heath, A. 1987. *Water pollution and fish physiology.* CRC Press, Boca Raton, FL.

Hernberg, S. 1976. Biochemical, subclinical, and clinical responses to lead and their relation to different exposure levels as indicated by concentration of lead in blood. In *Effects and dose-response relationships of toxic metals*, ed. G. Norberg. Elsevier, Amsterdam, 404.

Hinton, D.E., and J.A. Couch. 1984. Pathobiological measures of marine pollution effects. In *Concepts in marine pollution measurements*, ed. H.H. White, pp. 7-32. Maryland Sea Grant College, College Park, MD.

Hinton, D., J. Couch, S. Teh, and L. Courtney. 1988. Cytological changes during progression of neoplasia in selected fish species. In *Aquatic life toxicology, toxic chemicals and aquatic life: Research and management*, ed. D. Malins, A. Jensen, and M. Moore. Elsevier Science Publishers.

Holeton, G. 1972. Gas exchange in fish with and without hemoglobin. *Respir. Physicol.* 14: 142.

Hose, J.E., J.N. Cross, S.G. Smith, and D. Diehl. 1989. Reproductive impairment in a fish inhabiting a contaminated coastal environment off southern California. *Environ. Poll.* 57: 139-148.

Howard, D.W., and C.S. Smith. 1983. *Histological techniques for marine bivalve molluscs.* NOAA Technical Memorandum. NMFS-F/NEC-25. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Woods Hole, MA.

Hunn, J. 1988. *Field assessment of the effects of contaminants on fishes.* Biological Report 88, Fish and Wildlife Service, U.S. Department of the Interior, Columbia, MO.

Jerne, N., and A. Nordin. 1963. Plaque formation in agar by single antibody-producing cells. *Science* 140: 405.

Jimenez, B., A. Oikari, S. Adams, D. Hinton, and J. McCarthy. 1990. Hepatic enzymes as biomarkers of environmental, physiological and toxicological variables. In *Biomarkers of environmental contamination*, ed. J. McCarthy and L. Shugart, pp. 123-142. CRC Press, Boca Raton, FL.

Johnson, R., and H. Bergman. 1984. Use of histopathology in aquatic toxicology: A critique. In *Contaminant effects on fisheries*, ed. V. Cairns, P. Hodson, J. Nriagu. John Wiley and Sons, New York, NY.

Kleinow, K., M. MeLancon, and J. Lech. 1987. Biotransformation and induction: Implications for toxicity, bioaccumulation and monitoring of environmental xenobiotics in fish. *Environ. Health Perspect.* 71: 105-119.

Klingerman, A.D. 1982. Fishes as biological detectors of the effects of genotoxic agents. In *Mutagenicity, new horizons in genetic toxicology*, ed. J. Heddle. Academic Press.

Klontz, G. 1985. Diagnostic methods in fish diseases: Present status and needs. In *Fish and shellfish pathology*, ed. A. Ellis. Academic Press Inc.

Landolt, M.L., and R.M. Kocan. 1983. Fish cell cytogenetics: A measure of the genotoxic effects of environmental pollutants. In *Aquatic toxicology*, ed. J. Nriagu. John Wiley and Sons, New York, NY.

Larson, A., C. Haux, and M. Sjobeck. 1985. Fish physiology and metal pollution: Results and experiences from laboratory and field studies. *Ecotoxicol. Environ. Saf.* 9: 250.

Lech, J., M. Vodicnik, and C. Elcombe. 1982. Induction of mono-oxygenase activity in fish. In *Aquatic toxicology*, ed. L. Weber, pp. 107-148. Raven Press.

Luna, L. 1968. *Manual of histologic staining methods of the Armed Forces Institute of Pathology*. McGraw-Hill Book Company, The Blakiston Division, New York, NY.

Malins, D.C., and A. Jensen. 1988. *Aquatic toxicology*. Elsevier Science Publishers, Amsterdam.

Matthews, E., J. Warinner, and B. Weeks. 1990. Assays of immune function in fish macrophages. In *Techniques in fish immunology*, ed. J. Stolen, T. Fletcher, D. Anderson, B. Roberson, and W. van Muiswinkel. SOS Publications, Fair Haven, NJ.

May, E.B., M.J. Garreis, and M.M. Lipsky. 1985. *Histological markers of environmental effect.* 4th Symposium on Coastal and Ocean Management.

McCarthy, J.F. 1990. Concluding remarks: Implementation of a biomarker-based environmental monitoring program. In *Biomarkers of environmental contamination*, ed. J. McCarthy and L. Shugart. Lewis Publishers, Boca Raton, FL.

McCarthy, J.F., and L.R. Shugart, eds. 1990. *Biomarkers of environmental contamination.* Lewis Publishers, Boca Raton, FL.

McLea, D., and M. Gordon. 1977. Leucocrit: A simple haematological technique for measuring acute stress in salmonid fish, including stressful concentrations of pulpmill effluent. *J. Fish Res. Bd. Can.* 34: 2164.

Meyers, T.R., and J.D. Hendricks. 1985. Histopathology. In *Fundamentals of aquatic toxicology: Methods and applications*, ed. G.M. Rand and S.R. Petrocelli, pp. 283-331. Hemisphere Publishing Co., New York.

Mix, M.C. 1986. Cancerous diseases in aquatic animals and their association with environmental pollutants: A critical literature review. *Mar. Environ. Res.* 20 (1&2):1-141.

Myers, M.S., J.T. Landahl, M.M. Krahn, and B. B. McCain. 1991. Relationships between hepatic neoplasms and related lesions and exposure to toxic chemicals in marine fish from the U.S. West Coast. *Environ. Health Perspect.* 90:7-16.

Neff, J.M. 1985. *Use of biochemical measurements to detect pollutant-mediated damage to fish.* American Society for Testing and Materials, Special Technical Publication 854: 155-181.

Nielson, L.A., and D.L. Johnson, eds. 1984. *Fisheries techniques.* American Fisheries Society, Bethesda, MD.

Overstreet, R.M. 1988. Aquatic pollution problems, southeastern U.S. coasts: Histopathological indicators. *Aquat. Toxicol.* 11:213-239.

Passino, D.R. 1984. Biochemical indicators of stress in fishes: An overview. In *Aquatic toxicology*, ed. J. Nriagu. John Wiley and Sons, New York, NY.

Patton, J., and J. Couch. 1984. Can tissue anomalies that occur in marine fish implicate specific pollutant chemicals? In *Concepts in marine pollution measurements*, ed. H. White. Maryland Sea Grant College, University of Maryland.

Payne, J., L. Fancey, A. Rahimtula, and E. Porter. 1987. Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. *Comp. Biochem. Physiol.* C86: 233-245.

Peters, G., H. Delventhal, and H. Klinger. 1980. Physiological and morphological effects of social stress in the eel, *Anguilla anguilla*. *L. Art. Fisch. Wiss.* 30: 157.

Pickering, A. 1981. *Stress and fish.* Academic Press.

Roberts, R. J. 1989. *Fish pathology.* Ballmore Tindall, London, England.

Rowley, A. 1990. Collection, separation and identification of fish leucocytes. In *Techniques in fish immunology*, ed. J. Stolen, T. Fletcher, D. Anderson, B. Roberson, and W. van Muiswinkel. SOS Publications, Fair Haven, NJ.

Sanders, B. 1990. Stress proteins. In *Biomarkers of environmental contamination*, ed. J. McCarthy and L. Shugart. CRC Press, Boca Raton, FL.

Schmid, W. 1982. Chapter 36 in *Chemical mutagens: Principles and methods for their detection*, ed. A. Hollaender. Plenum Press.

Shugart, L.R. 1990. Biological monitoring: Testing for genotoxicity. In *Biomarkers of environmental contamination*, ed. J. McCarthy and L. Shugart. CRC Press, Boca Raton, FL.

Sindermann, C.J. 1983. An examination of some relationships between pollution and disease. *Rapp. P.-V. Reun. Cons. Int. Explor. Mer.* 182: 37-43.

Sindermann, C.J. 1990. *Principal diseases of marine fish and shellfish.* Vol. 1 and 2. Academic Press, San Diego, CA.

Snieszko, S. 1974. The effects of environmental stress on outbreaks of infectious diseases of fish. *J. Fish Biol.* 6: 197-208.

Sorenson, E.M. 1991. *Metal poisoning in fish.* CRC Press, Boca Raton, FL.

Sorenson, E.M.B., and N.K.R. Smith. 1981. Hemosiderin granules: Cytotoxic response to arsenic exposure in channel catfish, *Ictalurns punctatus*. *Bull. Environ. Contam. Toxicol.* 27: 645-653.

Sparks, A.K. 1985. *Synopsis of invertebrate pathology exclusive of insects.* Elsevier Science Publishers, Amsterdam.

Stedman's Medical Dictionary. 1982. 24th ed. Williams & Wilkins, Baltimore, MD.

Stegeman, J.J., and J.J. Lech. 1991. Cytochrome P-450 systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. *Environ. Health Perpect.* 90:101-116.

Stegeman, J., K. Renton, B. Woodin, Y. Zhang, and R. Addison. 1990. Experimental and environmental induction of cytochrome $P_{450}E$ in fish from Bermuda waters. *J. Exp. Mar. Biol. Ecol.* 138: 49-67.

Stegeman, J., B. Woodin, A. Goksoyr. 1988. Apparent cytochrome P-450 induction as an indication of exposure to environmental chemicals in the flounder *Platichthys flesus. Marine Ecol. Prog. Serv.* 46: 55-60.

Sumner, B.E.H. 1988. *Basic histochemistry.* John Wiley and Sons, New York, NY.

Taber's Cyclopedic Medical Dictionary. 1985. F. A. Davis Company, Philadelphia, PA.

Thomas, P. 1990. Molecular and biochemical responses of fish to stressors and their potential use in environmental monitoring. *Proceedings American Fisheries Society Symposium* 8: 9-28, Washington, DC.

Turgeon, D.D., S.B. Bricker, and T.P. O'Connor. In press. National status and trends program: Chemical and biological monitoring of U.S. coastal waters. In *Ecological indicators*, Vol. 1, ed. D.H. McKenzie and D.E. Hyatt. Elsevier Applied Science, Essex, England.

USEPA. 1985. *Methods for measuring the acute toxicity of effluents to freshwater and marine organisms.* 3d. ed. EPA/600/4-85/013. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

USEPA. 1986. *Proceedings and summary of the workshop on finfish as indicators of toxic contamination.* July 27-28, 1986, Arlie, VA. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1987. *Guidance for conducting fish liver histopathology studies during 301(h) monitoring.* EPA 430/09-87-004. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1987. *Bioaccumulation monitoring guidance: Strategies for sample replication and compositing.* Vol. 5. EPA 430/9-87-003. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1987. *Technical support document for ODES statistical power analysis.* EPA 430/9-87-005. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1988. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms.* EPA/600/4-87/028. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

USEPA. 1989. *Rapid bioassessment protocols for use in streams and rivers.* EPA 444/4-89-001. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1990. *Three year assessment of reproductive success in winter flounder,* Pseudopleuronectes americanus *(Walbaum), in Long Island Sound, with comparisons to Boston Harbor: 1986-1988.* I. Reproductive cycle: Vitellogenin. II. Comparative reproductive success: Biology, biochemistry, chemistry. III. Comparative embryo development and mortality. Final report. U.S. Environmental Protection Agency, Regions I, II. Long Island Sound Project.

Vogelbein, W., J. Fournie, P. VanVeld, and R. Huggett. 1990. Hepatic neoplasms in the mummichog *Fundulus heteroclitus* from a creosote-contaminated site. *Cancer Res.* 50: 5978-5986.

Warinner, J., E. Mathews, and B. Weeks. 1988. Preliminary investigations of the chemiluminescent response in normal and pollutant-exposed fish. *Mar. Environ. Res.* 24: 281-284.

Wedemeyer G., R. Gould, and W. Yasutake. 1983. Some potentials and limits of the leucocrit test as a fish health assessment method. *J. Fish Biol.* 23: 711.

Wedemeyer, G., and W. Yasutake. 1977. *Clinical methods for the assessment of the effects of environmental stress on fish health.* Technical paper 89. U.S. Fish and Wildlife Service, U.S. Department of the Interior, Washington, DC.

Weeks, B., R. Huggett, J. Warinner, and E. Mathews. 1990. Macrophage responses of estuarine fish as bioindicators of toxic contamination. In *Biomarkers of environmental contamination*, ed. J. McCarthy and L. Shugart. CRC Press, Boca Raton, FL.

Weeks, B., A. Keisler, J. Warinner, and E. Mathews. 1987. Preliminary evaluation of macrophage pinocytosis as a technique to monitor fish health. *Mar. Environ. Res.* 22: 205-213.

Weeks, B., and J. Warinner. 1984. Effects of toxic chemicals on macrophage phagocytosis in two estuarine fishes. *Mar. Environ. Res.* 14: 327-335.

Weeks, B., and J. Warinner. 1986. Functional evaluation of macrophages in fish from a polluted estuary. *Vet. Immunol. Immun.* 12: 313-320.

Weeks, B., J. Warinner, P. Mason, and D. McGinnis. 1986. Influence of toxic chemicals on the chemotactic response of fish macrophages. *J. Fish Biol.* 28: 653-658.

Weis, J.S., P. Weis, and E.J. Zimmerer. 1990. Potential utility of fin regeneration in testing sublethal effects of wastes. In *Oceanic processes in marine pollution.* Vol. 6, Physical and chemical processes: Transport and transportation, ed. D. Baumgortner and I. Duedall. Krieger Pub. Co.

West, G. 1990. Methods of assessing ovarian development in fishes: A review. *Aust. J. Mar. Freshwater Res.* 41: 199-222.

Wolke, R., C. George, and V. Blazer. 1984. Pigmented macrophage accumulations (MMC;PMB): Possible monitor of fish health. In *Parasitology and pathology of marine organisms in the world ocean*, ed: W. Hargis, p. 93. NOAA Technical Report NMFS 25, p. 93.

Wydoski, R., and G. Wedemeyer. 1976. Physiological responses of fish: Problems and progress toward use in environmental monitoring. In *Aquatic toxicology*, ed V. Cairns, P. Hodson, and J. Nriagu. John Wiley and Sons, New York, NY.

Yevich, P.P., and C.A. Barszcz. 1980. Preparation of aquatic animals for histopathological examination. In *International mussel watch*, Appendix 6-13, pp. 212-220. U. S. National Academy of Sciences, Washington, DC.

Yevich, P.P., and C.A. Barszcz. 1983. Histopathology as a monitor for marine pollution: Results of histopathological examinations of the animals collected for the 1976 Mussel Watch Program. *Rapp. P. -v. Reun. Cons. Int. Explor. Mer* 182:96-102.

FISH POPULATIONS

Armstrong, N.E., P.N. Storrs, and H.F. Ludwig. 1970. Ecosystem-pollution interactions in San Francisco Bay. *J. Water Poll. Control Fed.*

Anderberg, M.R. 1973. *Cluster analysis for applications.* Academic Press, New York, NY.

Bechtel, T.J., and B.J. Copeland. 1970. Fish species diversity indices as indicators of pollution in Galveston Bay, Texas. *Contrib. in Mar. Sci.* 15:103-132.

Boesch, D.F. 1977. *Application of numerical classification in ecological investigations of water pollution.* EPA 600/3-77-033. U.S. Environmental Protection Agency, Office of Research and Development, Corvallis, OR.

Bond, C.E. 1979. *Biology of fishes.* Sanders College Publishing, Philadelphia, PA.

Cailliet, G.M., M.S. Love, and A.W. Ebeling. 1986. *Fishes: A field and laboratory manual on their structure, identification, and natural history.* Wadsworth Publishing Company, Belmont, CA.

Clifford, H.T., and W. Stephenson. 1975. *An introduction to numerical classification.* Academic Press, New York, NY.

Curtis, M.A., and G.H. Peterson. 1978. Size-class heterogeneity with spatial distribution of subartic marine benthos populations. *Astarte* 10:103-105.

Cushing, D.J. 1975. *Marine ecology and fisheries.* Cambridge University Press, Cambridge, UK.

Ferraro, S.P., F.A. Cole, W.A. DeBen, and R.C. Swartz. 1989. Power-cost efficiency of eight macrobenthic sampling schemes in Puget Sound, Washington, USA. *Can. J. Fish. Aquat. Sci.* 46: 2157-2165.

Fredette, T.J., D.A. Nelson, T. Miller-Way, J.A. Adair, V.A. Sotler, J.E. Clausner, E.B. Hands, and F.J. Anders. 1989. *Selected tools and techniques for physical and biological monitoring of aquatic dredged material disposal sites.* Final report. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Gauch, H.G. 1982. *Multivariate analysis in community ecology.* Cambridge University Press, Cambridge, UK.

Green, R.H. 1979. *Sampling design and statistical methods for environmental biologists.* John Wiley and Sons, New York, NY.

Green, R.H. 1984. Statistical and nonstatistical considerations for environmental monitoring studies. *Environ. Monit. Assess.* 4: 293-301.

Hurlbert, S.H. 1971. The nonconcept of species diversity: A critique and alternative parameters. *Ecol.* 52: 577-586.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* 54: 187-211.

Margalef, R. 1969. Diversity and stability: A practical proposal and a model of interdependence. In Diversity and stability in ecological systems, ed. G.M. Woodwell and H.H. Smith. *Brookhaven Symp. Biol.* 22:25-37.

Nielson, L.A., and D.L. Johnson, eds. 1984. *Fisheries techniques.* American Fisheries Society, Bethesda, MD.

Pielou, E.C. 1966, The measurement of diversity in different types of biological collections. *J. Theoret. Biol.* 13:131-144.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. *Bull. Fish. Res. Bd. Can.* 191:382 pp.

Romesburg, H.C. 1984. *Cluster analysis for researchers.* Lifetime Learning Publications, Belmont, CA.

Rothschild, B.J. 1986. *Dynamics of marine fish populations.* Harvard University Press, Cambridge, MA.

Self, S.G., and R.H. Mauritsen. 1988. Power/sample size calculations for generalized linear models. *Biometrics* 44: 79-86.

Sneath, P.H.A., and R.R. Sokal. 1973. *Numerical taxonomy: The principles and practices of numerical classification.* Freeman, San Francisco, CA.

Swartz, R.C., D.W. Schultz, G.R. Ditsworth, W.A. DeBen, and F.A. Cole. 1985. Sediment toxicity, contamination, and macrobenthic communities near a large sewage outfall. In *Validation and predictability of lboratory mthods for asessing the fate and effects of contaminants in aquatic ecosystems*, ed. T.T. Boyle. American Society for Testing and Materials (ASTM), Philadelphia, PA.

Tsai, Chu-Fa. 1968. Effects of chlorinated sewage effluents on fishes in Upper Patuxent River, Maryland. *Chesapeake Sci.* 9:83-93.

USEPA. 1978. *Use of small otter trawls in coastal biological surveys.* EPA 600/3-78-083. U.S. Environmental Protection Agency, Office of Research and Development, Corvallis, OR.

USEPA. 1982. *Design of 301(h) monitoring programs for municipal wastewater discharges to marine waters.* U.S. Environmental Protection Agency, Office of Water, Washington, DC.

USEPA. 1985. *Recommended biological indices for 301(h) monitoring programs.* EPA 430/9-86-002. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1986-1991. *Recommended protocols for measuring selected environmental variables in Puget Sound.* Looseleaf. U.S. Environmental Protection Agency, Region 10, Puget Sound Estuary Program, Seattle, WA.

USEPA. 1987. *Technical support document for ODES statistical power analysis.* EPA 430/9-87-005. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1988. *ODES data brief: Use of numerical classification.* U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1990. *Environmental Monitoring and Assessment Program: Ecological indicators.* EPA 600/3-90-060. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

Whipple, J.A., M. Jung, R.B. MacFarlane, and R. Fischer. 1984. *Histopathological manual for monitoring health of striped bass in relation to pollutant burdens.* NOAA Tech. Mem. NMFS, U.S. Department of Commerce, NOAA TM-NMFS-SWFC-46.

PLANKTON: BIOMASS, PRODUCTIVITY, AND COMMUNITY STRUCTURE/FUNCTION

Abaychi, J.K., and J.P. Riley. 1979. The determination of phytoplankton pigments by high-performance liquid chromatography. *Anal. Chim. Acta.*

Ahlstrom, E. 1969. *Recommended procedures for measuring the productivity of plankton standing stock and related oceanic properties.* National Academy of Science.

APHA. 1989. American Public Health Association, American Water Works Association, and Water Pollution Control Federation. *Standard methods for the examination of water and wastewater.* 17th ed. American Public Health Association, Washington, DC.

ASTM. 1979. American Society for Testing and Materials. Water. In *Annual book of ASTM standards*, Part 31. Amer. Soc. Test. Mat., Philadelphia, PA.

Azam, F., T. Fenchel, J.G. Field, J.S. Gray, L.A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water column microbes in the sea. *Mar. Ecol. Prog. Ser.* 10:257-263.

Beers, J.R. 1978. Pump sampling. In *Monographs on oceanographic methodology.* 6. Phytoplankton manual, pp. 41-49. United Nations Educational, Scientific, and Cultural Organization, Paris, France.

Beers, J.R., G.L. Stewart, and J.D.H. Strickland. 1967. A pumping system for sampling small plankton. *J. Fish. Res. Board Can.* 24:1811-1818.

Boesch, D.F. 1977. *Application of numerical classification in ecological investigations of water pollution.* Rep. no. 600/3-77-033. U.S. Environmental Protection Agency, Corvallis, OR.

Brown, L.M., B.T. Hargrave, and M.D. Mackinnon. 1981. Analysis of chlorophyll *a* in sediments by high-pressure liquid chromatography. *Can. J. Fish. Aquat. Sci.* 38:205-214.

Burnison, B.K. 1980. Modified dimethyl sulfoxide (DMSO) extraction for chlorophyll analysis of phytoplankton. *Can. J. Fish. Aquat. Sci.* 37:729-733.

Burrell, V.G., W.A. Van Engel, and S.G. Hummel. 1974. A new device for subsampling plankton species. *J. Cons. Perm. Int. Explor. Mer.* 35:364-366.

Chesapeake Executive Council, 1988. *Living resources monitoring plan.* Chesapeake Bay Program, Annapolis, MD.

Clifford, H.T., and W. Stephenson. 1975. *An introduction to numerical classification.* Academic Press, San Francisco, CA.

Cochran, W.G. 1963. *Sampling techniques.* 2d ed. John Wiley and Sons, Inc., New York, NY.

Cochran, W.G. 1977. *Sampling techniques.* 3d ed. John Wiley and Sons, Inc. New York, NY.

Copeland, B.J. 1966. Effects of industrial waste on the marine environment. *J. Water Poll. Cont. Fed.* 38:1000-1010.

Copeland, B.J., and D.E. Wohlschlag. 1968. Biological responses to nutrients-eutrophication: Saline water considerations In *Advances in water quality improvement*, ed. E.F. Gloyna and W.W. Eckenfelder, pp. 65-82. Univ. Tex. Press, Austin, TX.

Cooley, W.W., and P.R. Lohnes. 1971. *Multivariate data analysis.* John Wiley and Sons, Inc. New York, NY.

Crossman, J.S., R.L. Kaisler, and J. Cairns, Jr. 1974. The use of cluster analysis in the assessment of spills of hazardous materials. *Amer. Midland Natur.* 92:94-114.

D'Elia, C.F., K.L. Webb, D.V. Shaw, and C.W. Keefe. 1986. *Methodological comparisons for nitrogen and chlorophyll determinations in estuarine water samples.* Submitted to the Power Plant Siting Program, Maryland Department of Natural Resources, Annapolis, MD, and Chesapeake Bay Liaison Office, U.S. Environmental Protection Agency, Annapolis, MD.

Dunn, O.J. 1964. Multiple comparisons using rank sums. *Technometric* 6(3):241-252.

Garside, C., and J.P. Riley. 1969. A thin-layer chromatographic method for the determination of plant pigments in sea water and cultures. *Anal. Chim. Acta* 46:179-191.

Gauch, H.G., and R.H. Whittaker. 1972. Comparison of ordination techniques. *Ecology* 53:868-875.

Gibbs, C.F. 1979. Chlorophyll *a* and 'phaeo-pigments.' *Aust. J. Mar. Freshwater Res.* 30:596-606.

Gieskes, W.W.C., and G.W. Kraay. 1983. Dominance of Cryptophyceae during the phytoplankton spring bloom in the central North Sea detected by HPLC analysis of pigments. *Mar. Biol.* 75:179-185.

Goeyens, L., E. Post, F. Deharis, A. Vandenhoudt, and W. Baeyens. 1982. The use of high pressure liquid chromatography with fluorimetric detection for chlorophyll a determination in natural extracts of chloropigments and their degradation products. *Intern. J. Environ. Anal. Chem.* 12:51-63.

Gordon, D.C., and W.H. Sutcliffe, Jr. 1974. Filtration of seawater using silver filters for particulate nitrogen and carbon analysis. *Limnol. Oceanogr.* 19:989-993.

Grasshoff, K., M. Ehrhardt, and K. Kremling. 1973. *Methods of seawater analysis*, 2d ed. Verlag-Chimie, Weinheim.

Green, R.H. 1979. *Sampling design and statistical methods for environmental biologists.* John Wiley and Sons, New York, NY.

Green, R.H. 1980. Multivariate approaches in ecology: The assessment of ecologic similarity. *Ann. Rev. Ecol. Syst.* 11:1-14.

Green, R.H. 1984. Some guidelines for the design of biological monitoring programs in the marine environment. In *Concepts of marine pollution measurements*, ed. H.H. White, pp. 233-245. University of Maryland Sea Grant, College Park, MD.

Green, R.H., and G.L. Vascotto. 1978. A method for the analysis of environmental factors controlling patterns of species composition in aquatic communities. *Water Res.* 12:583-590.

Heck, K.L., Jr., and R.J. Horwitz. 1984. Statistical analysis of sampling data to assess impact on marine environments. In *Concepts of marine pollution measurements*, ed. H.H. White, pp. 233-245. University of Maryland Sea Grant, College Park, MD.

Hollander, M., and D.A. Wolfe. 1973. *Nonparametric statistical methods.* John Wiley and Sons, New York, NY.

Hurlbert, S.H. 1971. The nonconcept of species diversity: A critique and alternative parameters. *Ecol.* 52:577-586.

Inskeep, W.P., and P.R. Bloom. 1985. Extinction coefficients of chlorophyll a and b in N, N-dimetylformamide and 80% acetone. *Plant Physiol.* 77:483-485.

Jacobs, F., and G.C. Grant. 1978. *Guidelines for zooplankton sampling in quantitative baseline and monitoring programs.* Rep. No. 600/3-7-78-026. U.S. Environmental Protection Agency, Corvallis, OR.

Jeffrey, S.W., and G.M. Hallegraeff. 1980. Studies of phytoplankton species and photosynthetic pigments in a warm core eddy of the East Australian Current. I. Summer populations. *Mar. Ecol. Prog. Ser.* 3:285-295.

Jeffrey, S.W., and G.F. Humphrey. 1975. New spectrophotometric equations for determining chlorophyll *a*, *b*, *c1* and *c2* in higher plants, algae and natural phytoplankton. *Biochem. Physicol. Pflanzen.* 167:191-194.

Jeffrey, S.W., M. Sielicki, and F.T. Hazo. 1975. Chloroplast pigment patterns in dinoflagellates. *J. Physicol.* 11:374-384.

Johnson, B.D., and P.J. Wangersky. Seawater filtration: Particle and impaction considerations. *Limnol. Oceanogr.* 30(5):966-972.

Knight, R., and R.F.C. Mantoura. 1985. Chlorophyll and carotenoid pigments in Foraminifera and their symbiotic algae: analysis by high performance liquid chromatography. *Mar. Ecol. Prog. Ser.* 23:241-249.

Lorenzen, C.J. 1966. A method for the continuous measurement of in vivo chlorophyll concentration. *Deep-Sea Res.* 13:223-227.

Lorenzen, C.J. 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. *Limnol. Oceanogr.* 12: 343-346.

Magnien, R.E. 1986. *A comparison of estuarine water quality chemistry analysis on the filtrate from two types of filters.* Final draft. Maryland Office of Environmental Programs, Ecological Modeling and Analysis Division, Technical Report. Baltimore, MD.

Mantoura, R.F.C., and C.A. Llewellyn. 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. *Anal. Chem. Acta* 151:297-314.

McEwen, G.F., M.W. Johnson, and T.R. Folsom. 1954. A statistical analysis of the performance of the Folson plankton sample splitter based upon test observations. *Arch. Meteorol. Geophys. Biolkimatoll., Ser. A. Meteorol. Geophys.* 7:502-527.

McGowan, J.A., and D.M. Brown. 1966. *A new opening-closing paired zooplankton net.* Univ. Calif. Scripps Inst. Oceanogr. (Ref. 66-23).

McGowan, J.A., and V.J. Fraundorf. 1966. The relationship between size of net used and estimates of zooplankton diversity. *Limnol. Oceanogr.* 11: 456-469.

Moran, R., and D. Porath. 1980. Chlorophyll determination in intact tissues using N,N-dimethylformamide. *Plant Physiol.* 65:478-479.

Odum, H.T., R.P. Cuzan du Rest, R.J. Beyers, and C. Allbaugh. 1963. Diurnal metabolism, total phosphorus, Ohle anomaly, and zooplankton diversity of abnormal marine ecosystems of Texas. *Publs. Inst. Mar. Sci. Tex.* 9:404-453.

Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. *A manual of chemical and biological methods for seawater analysis.* Pergamon Press, New York, NY.

Patten, B.C. 1962. Species diversity in net phytoplankton of Raritan Bay. *J. Mar. Res.* 20:57-75.

Pielou, E.C. 1966. The measurement of diversity in different types of biological collections. *J. Theoret. Biol.* 13: 131-144.

Pielou, E.C. 1970. *An introduction to mathematical ecology.* Wiley-Interscience, New York, NY.

Pielou, E.C. 1977. *Mathematical ecology.* Wiley-Interscience, New York, NY.

Pomeroy, L.R. 1984. Significance of microorganisms in carbon and energy flow in marine ecosystems. In *Current perspectives in microbial ecology*, ed. M.J. Klug and C. A. Reddy. pp. 405-411. American Society for Microbiology, Washington, DC.

Puget Sound Monitoring Management Committee. 1988. *Puget Sound Ambient Monitoring Program.* Puget Sound Water Quality Authority, Seattle, WA.

Saila, S.B., D. Chen, V.J. Pigoga, and S.D. Pratt. 1984. Comparative evaluation of some diversity measures for assessing environmental changes in an estuarine community. In *Concepts of marine pollution measurements*, ed. H.H. White, pp. 217-233. University of Maryland Sea Grant, College Park, MD.

SCORE. 1966. *Monographs on oceanographic methodology. Vol. 1. Determination of photosynthetic pigments in seawater.* UNESCO Press, Paris, France.

Seely, G.R., M.J. Duncan, and W.E. Vidaver. 1972. Preparative and analytical extraction of pigments from brown algae with dimethyl sulfoxide. *Mar. Biol.* 12:184-188.

Sherr, B., and E. Sherr. 1983. Enumeration of heterotrophic microprotozoa by epifluorescence microscopy. *Est. Cstl. Shelf Sci.* 16:1-7.

Shioi, Y., R. Fukae, and T. Sasa. 1983. Chlorophyll analysis by high-performance liquid chromatography. *Biochem. Biophy. Acta* 722:72-79.

Shoaf, W.T., and B.W. Lium. 1976. Improved extraction of chlorophyll a and b from algae using dimethyl sulfoxide. *Limnol. Oceanogr.* 21: 926-928.

Siegel, S. 1956. *Nonparametric statistics for the behavioral sciences.* McGraw-Hill Book Co., New York, NY.

Smith, R.W., and C.S. Greene, 1976. Biological communities near submarine outfall. *J. Water Poll. Control Fed.* 48(8):1894-1912.

Smith, W., V.R. Gibson, L.S. Brown-Leger, and J.F. Grassle. 1979. Diversity as an indicator of pollution: Cautionary results from microcosm experiments. In *Ecological diversity in theory and practice*, ed. Graale et al., pp. 269-277. Internat. Coop. Publ. House, Fairland, MD.

Sneath, P., and R. Sokal. 1973. *Numerical taxonomy.* W.H. Freeman and Co., San Francisco, CA.

Snedecor, G.W., and W.G. Cochran. 1967. *Statistical methods.* 6th ed. The Iowa State University Press.

Sokal, R.R., and F.J. Rohlf. 1969. *Biometry.* W.H. Freeman and Co., San Francisco, CA.

Speziale, B.J., S.P. Schreiner, P.A. Giammatteo, and J.E. Schindler. 1984. Comparison of N, N-dimethylformamide, dimethyl sulfoxide, and acetone for extraction of phytoplankton chlorophyll. *Can. J. Fish. Aquat. Sci.* 41:1519-1522.

Sprules, W.G. 1977. Crustacean zooplankton communities as indicators of limnological conditions: an approach using principal component analysis. *J. Fish Res. Board Can.* 34:962-975.

Stauffer, R.E., G.F. Lee, and D.E. Armstrong. 1979. Estimating chlorophyll extraction biases. *J. Fish. Res. Board Can.* 36:142-157.

Stofan, P.F., and G.C. Grant. 1978. *Phytoplankton sampling in quantitative baseline and monitoring programs.* Rep. No. 600/3-78-025. U.S. Environmental Protection Agency, Corvallis, OR.

Strickland, J.D.H., and T.R. Parsons. 1968. A practical handbook of seawater analysis. Fish. Res. Board Can. Bull. No. 167.

Strickland, J.D.H., and T.R. Parsons. 1972. A practical handbook of seawater analysis. *Bull. Fish. Res. Board. Can.*

Swartz, R.C., D.W. Schultz, G.R. Ditsworth, W.A. DeBen, and F.A. Cole. 1985. Sediment toxicity, contamination, and macrobenthic communities near a large sewage outfall. In *Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystems*, ed. T.T. Boyle. pp. 152-175. American Society for Testing and Materials (ASTM), Philadelphia, PA.

Throndsen, J. 1978. *Preservation and storage. In Monographs on oceanographic methodology. 6. Phytoplankton manual*, pp. 69-71. United Nations Education, Scientific, and Cultural Organization, Paris, France.

UNESCO. 1968. *Monographs on oceanographic methodology. 2. Zooplankton sampling.* United Nations Education, Scientific, and Cultural Organization, Paris, France.

UNESCO. 1973. *Monographs on oceanographic methodology. 3. A guide to the measurement of marine primary production under some special conditions.* United Nations Education, Scientific, and Cultural Organization, Paris, France.

UNESCO. 1978. *Monographs on oceanography methodology. 6. Phytoplankton manual.* United Nations Education, Scientific, and Cultural Organization, Paris, France.

USEPA. 1979. *Handbook for analytical quality control in water and wastewater laboratories.* U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, EPA-600/4-79-019, Cincinnati, OH.

USEPA. 1982. *Design of 301(h) monitoring programs for municipal wastewater discharges to marine waters.* U.S. Environmental Protection Agency, Office of Water, Washington, DC.

USEPA. 1985. *Interim guidance on quality assurance/quality control (QA/QC) for the estuarine field and laboratory methods.* U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1987. *Technical support document for ODES statistical power analysis.* EPA 430/9-87-005. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1989. *Chesapeake Bay basin monitoring program atlas* (Vols. 1 and 2). U.S. Environmental Protection Agency, Chesapeake Bay Liaison Office, Annapolis, MD.

USEPA. 1989. *Compendium of methods for marine and estuarine environmental studies.* Draft. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Venrick, E.L. 1978. Water-bottles. In *Monographs on oceanographic methodology. 6. Phytoplankton manual*, pp. 33-40. United Nations Education, Scientific, and Cultural Organization, Paris, France.

Venrick, E.L. 1978. The implications of subsampling. In *Monographs on oceanographic methodology. 6. Phytoplankton manual*, pp. 75-87. United Nations Education, Scientific, and Cultural Organization, Paris, France.

Wiebe, P.H., and W.R. Holland. 1968. Plankton patchiness: Effects on repeated net tows. *Limno. Oceanogr.* 13:315-321.

Williams, W.T. 1971. Principles of clustering. *An. Rev. Ecolo. Syst.* 2:303-326.

Winer, B.J. 1971. *Statistical principles in experimental design.* McGraw-Hill Book Company, New York, NY.

Wood, A.M. 1979. Chlorophyll *a:b* in marine planktonic algae. *J. Phycol.* 15: 330-332.

Wood, L.W. 1985. Chloroform-methanol extraction of chlorophyll *a*. *Can. J. Fish. Aquat. Sci.* 42: 38-43.

Yentsch, C.S., and D.W. Menzel. 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep-Sea Res.* 10:221-231.

Zar, J.H. 1974. *Biostatistical analysis.* Prentice-Hall, Inc., Englewood Cliffs, NJ.

HABITAT IDENTIFICATION METHODS

Abraham, B.J., and P.L. Dillon. 1986. *Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates. (Mid Atlantic)—Soft shell clam.* U.S. Fish and Wildlife Service, FWS/OBS-82/11.68. U.S. Army Corps of Engineers, TR EL-82-4.

Adamus, P.R. 1988. The FHWA/Adamus (WET) method for wetland functional assessment. In *The ecology and management of wetlands*, ed. D.D. Hook et al., pp. 128-133. Croom Helm Publishers.

Adamus, P.R., L.T. Stockwell, E.J. Clairain, Jr., M.E. Morrow, L.P. Rozas, and R.D. Smith. 1987. *Wetland evaluation technique (WET). Vol. I. Literature review and evaluation rationale.* U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS.

Adamus, P.R., E.J. Clairain, Jr., D.R. Smith, and R.E. Young. 1987. *Wetland evaluation technique (WET). Vol. II. Technical report Y-87.* U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS.

Aggus, L.R., and W.M. Bivin. 1982. *Habitat suitability index models: Regression models based on harvest of cool and coldwater fishes in reservoirs.* U.S. Fish and Wildlife Service, Biological Services Program, Washington, DC.

Anon. 1985. *Proposed policy and procedures for fish habitat management.* Department of Fisheries and Oceans, Ottawa, Ontario, Canada.

Beauchamp, R.B., ed. 1974. *Marine environment planning guide for the Hampton Roads/Norfolk naval operating area.* Spec. Pub. No. 250. Naval Oceanographic Office, Washington, DC.

Bain, M.B., and J.L. Bain. 1982. *Habitat suitability index model: Coastal stocks of striped bass.* Rep. Natl. Coastal Ecosystems Team, U.S. Fish. Wildlife Service, Rep. No. FWS/OBS 82/10.1, Washington, DC.

Chesapeake Executive Council. 1988. *Habitat requirements for Chesapeake Bay living resources.* Chesapeake Bay Program, Annapolis, MD.

Colwell, M.A., and L.W. Oring. 1988. Habitat use by breeding and migrating shorebirds in south central Saskatchewan. *Wilson Bulletin* 100(4):554-566.

Cowardin, L.M., V. Carter, F.C. Golet, and E.T. LaRoe. 1979. *Classification of wetland and deepwater habitats of the United States.* U.S. Fish and Wildlife Service, Office of Biological Services, Washington, DC.

Dee, N., J. Baker, N. Drobner, K. Duke, I. Whitman, and D. Fahrigner. 1973. Environmental evaluation system for water resources planning, *Water Res. Res.* 9(3):523-534.

Department of Fisheries and Oceans. 1984. *A fishery officer's guide for fish habitat management and protection.* Ottawa, Ontario, Canada.

Diaz, R.J., and C.P. Onuf, 1985. *Habitat suitability index models: Juvenile Atlantic croaker.* Revised. Biological reports of the U.S. Fish and Wildlife Service, Washington, DC.

Fay, C.W., R.J. Neves, and G.B. Pardue. 1983. *Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (mid-Atlantic) - Striped bass.* WWS/OBS 82/11.8. U.S. Fish and Wildlife Service, Washington, DC.

Hardy, J.D., Jr. 1978. *Development of fishes of the mid-Atlantic Bight: An atlas of the egg, larval and juvenile stages.* Vol. III. U.S. Department of the Interior, Fish and Wildlife Service, Biological Service Program. FWS/OBS-78/12.

Heinen, J.I., and R.A. Mead. 1982. *The application of remote sensing to site- and species-specific wildlife habitat analysis.* Technical Reports of Virginia Polytechnical Institute, RR-82-2, NFAP-292.

Johnson, G.D. 1978. *Development of fishes of the mid-Atlantic Bight. An atlas of egg, larval and juvenile stages.* Volume IV. Carrangidae through Ephippidae. U.S. Department of the Interior, Fish and Wildlife Service, Biological Services Program. FWS/OBS-78/12.

Johnson, H.B., B.F. Holland, Jr., and S.G. Keefe. 1977. *Anadromous fisheries research program, northern coastal area.* N.C. Div. Mar. Fish. Rep. No. AFCS-11.

Jones, P.W., F.D. Martin, and J.D. Hardy, Jr. 1978. *Development of fishes of the mid-Atlantic Bight. An atlas of egg, larval and juvenile stages.* Vol. I. U.S. Department of the Interior, Fish and Wildlife Service, Biological Service Program. FWS/OBS-78/12.

Klein, R., and J.C. O'Dell. 1987. *Physical habitat requirement for fish and other living resources inhabiting class I and II waters.* Internal document. Maryland Department of Natural Resources, Tidewater Administration.

Lonard, R.I. , E.J. Clairain, Jr., R.T. Huffman, J.W. Hardy, L.D. Brown, P.E. Ballard, and J.W. Watts. 1981. *Analysis of methodologies for assessing wetlands value.* U.S. Water Resources Council, Washington, DC, and U.S. Army Corps of Engineers, Vicksburg, MS.

Lonard, R.I., and E.J. Clairain, Jr. 1986. Identification of methodologies for the assessment of wetland functions and values, In *Proceedings: National wetlands assessment symposium,* pp. 66-72. Association of State Wetland Managers, Inc., Portland, ME, June 17-20.

Marble, A.D., and M. Gross. 1984. A method for assessing wetland characteristics and values. *Landscape Plan.* 2:1-17.

Reppert, R.T., W. Siglero, E. Stakhiv, L. Messman, and C. Meyers. 1979. *Wetland values: Concepts and methods for wetlands evaluations.* IWR Research Report 79-R-1, U.S. Army Engineer Institute for Water Resources, Fort Belvoir, VA.

SCS Engineers. 1979. *Analysis of selected functional characteristics of wetlands.* Contract No. DACW73-78-R-0017, Reston, VA.

Segar, D.A. 1987. The Aquatic Habitat Institute: A new concept in estuarine pollution management. *Proceedings of the Tenth National Conference Estuarine and Coastal Management: Tools of the Trade.* New Orleans, Louisiana, 12-15 October 1986. Vol. 2, p. 501.

Solomon, R.D., B.K. Colbert, W.J. Hanses, S.E. Richardson, L.W. Ganter, and E.C. Vlachos. 1977. *Water Resources Assessment Methodology (WRAM)-Impact assessment and alternative evaluation.* Technical report Y-77-1, Environmental. Effects Laboratory, U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, MS.

State of Maryland Department of Natural Resources. Undated. *Environmental evaluation of coastal wetlands.* Draft. Tidal Wetlands Study, pp. 181-208.

Toole, C.L., R.A. Barnhart, and C.P. Onuf. 1987. *Habitat suitability index models: Juvenile English sole.* Biological reports of the U.S. Fish and Wildlife Service, Washington, DC.

U.S. Army Construction Engineering Research Lab. 1987. *Environmental gradient analysis, ordination and classification in environmental impact assessments.* Final report. Champaign, IL.

U.S. Army Engineer Division, Lower Mississippi Valley. 1980. *A habitat evaluation system for water resources planning.* U.S. Army Corps of Engineers, Lower Mississippi Valley Division, Vicksburg, MS.

USEPA. 1982. *Research on fish and wildlife habitat.* U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

USEPA. 1984. *Final ocean discharge criteria evaluation Navarin Basin OCS lease sale 83.* U.S. Environmental Protection Agency, Region 10, Seattle, WA.

USEPA. 1988. *Use of geographic information systems for wetlands protection.* U.S. Environmental Protection Agency, Office of Wetlands Protection, Washington, DC.

USFWS. 1978. Classification, inventory and analysis of fish and wildlife habitat: *Proceedings of a national symposium*, Phoenix, Arizona, January 24-27, 1977. U.S. Fish and Wildlife Service, Office of Biological Services, Washington, DC.

USFWS. 1980. *Habitat evaluation procedures (HEP) manual (102ESM).* U.S. Department of the Interior, Fish and Wildlife Service, Washington, DC.

USFWS. 1982. *Standards for the development of habitat suitability index models.* 103 ESM. U.S. Fish and Wildlife Service.

BIOACCUMULATION

Boehm, P.D. 1984. *The Status and Trends Program: Recommendations for design and implementation of the chemical measurement segment.* Workshop report. NOAA, Rockville, MD.

deBoer, J. 1988. Chlorobiphenyls in bound and non-bound lipids of fishes: Comparison of different extraction methods. *Chemosphere* 17: 1803-1810.

DiToro, D.M., J.D. Mahony, D.J. Hanson, K.J. Scott, A.R. Carlson, and G.T. Ankley. In press. *Acid volatile sulfide predicts the acute toxicity of cadimum and nickel in sediments.*

Ferraro, S.P., H. Lee, R.J. Ozretich, and D.T. Specht. 1990. Predicting bioaccumulation potential: A test of a fugacity-based model. *Arch. Environ. Contam. Toxicol.* 19: 386-394.

Fowler, S.W. 1982. Biological transfer and transport processes. In *Pollutant transfer and transport in the sea*, vol. 2, ed. G. Kullenberg. CRC Press, Boca Raton, FL.

Gardner, W.S., W.A. Frez, E.A. Cichocki, and C.C. Parrish. 1986. Micromethod for lipids in aquatic invertebrates. *Limnol. Oceanogr.* 30: 1099-1105.

Gardner, W.S., T.F. Nalepa, W.A. Frez, E.A. Cichocki, and P.F. Landrum. 1985. Seasonal patterns in lipid content of Lake Michigan macroinvertebrates. *Can. J. Fish. Aquat. Sci.* 42: 1827-1832.

Goldberg, E.D., V.T. Bowen, G.H. Farrington, J.H. Martin, P.L. Parker, R.W. Risebrough, W. Robertson, E. Schneider and E. Gamble. 1978. The mussel watch. *Environ. Conserv.* 5: 101-125.

Hansen, P.D., H. Von Westerhagen, and H. Rosenthal. 1985. Chlorinated hydrocarbons and hatching success in Baltic herring spring spawners. *Mar. Environ. Res.* 15: 59-76.

Hiatt, M.H. 1981. Analysis of fish and sediment for volatile priority pollutants. Anal. *Chem.* 53: 1541-1543.

Karickhoff, S.W., D.S. Brown, and T.A. Scott. 1979. Sorption of hydrophobic pollutants on natural sediments. *Wat. Res.* 13: 241-248.

Knezovich, J.P. and F.L. Harrison. 1987. A new method for determining the concentration of volatile organic compounds in sediment interstitial water. *Bull. Environ. Contam. Toxicol.* 38: 837-940.

Ladd, J.M., S.P. Hayes, M. Martin, M.D. Stephenson, S.L. Coale, J. Linfield, and M. Brown. 1984. *California state mussel watch: 1981-1983. Trace metals and synthetic organic compounds in mussels from California's coast, bays, and estuaries.* Biennial Report. Water Quality Monitoring Report No. 83-6TS. Sacramento, CA.

Lake, J.L., N.I. Rubinstein, and S. Parvignano. 1987. Predicting bioaccumulation: Development of a partitioning model for use as a screen tool in regulating ocean disposal of wastes. In *Fate and effects of sediment-bound chemicals in aquatic systems*, ed K.L. Dickson, A.W. Maki, and W.A. Brungs. Sixth Pellston Workshop, Florissant, CO.

Landrum, P.F., and J.A. Robbins. In press. Bioavailability of sediment-associated contaminants to benthic invertebrates. In *Sediments: Chemistry and toxicity of in-place pollutants*, ed. J.P. Giesy, R. Baudo, and H. Muntau. Lewis Publishers.

Niimi, A.J. 1983. Biological and toxicological effects of environmental contaminants in fish and their eggs. *Can. J. Fish. Aquat. Sci.* 40: 306-312.

Pearson, T.H., and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr. Mar. Biol. Ann. Rev.* 16: 229-311.

Phillips, D.J.H. 1980. *Quantitative aquatic biological indicators.* Applied Science Publ. Ltd., London, England.

Phillips, D.J.H., and D.S. Segar. 1986. Use of bio-indicators in monitoring conservative contaminants: Programme design imperatives. *Mar. Poll. Bull.* 17: 10-17.

Rubinstein, N.I., J.L. Lake, R.J. Pruell, H. Lee, B. Taplin, J. Heltshe, R. Bowen, and S. Parvignano. 1987. *Predicting bioaccumulation of sediment-associated organic contaminants: Development of a regulatory tool for dredged material evaluation.* Internal report. U.S. Environmental Protection Agency, Narragansett, RI.

Spies, R.B., D.W. Rice, and J. Felton. 1988. Effects of organic contaminants on reproduction of the starry flounder *Platichthys stellatus* in San Francisco Bay. *Mar. Biol.* 98: 181-189.

Tetra Tech. 1985. *Bioaccumulation monitoring guidance: Estimating the potential for bioaccumulation of priority pollutants and 301(h) pesticides discharged Into marine and estuarine waters.* Vol. 1. Tetra Tech, Inc., Bellevue, WA.

Tetra Tech. 1985. *Bioaccumulation monitoring guidance: Selection of target species and review of available bioaccumulation data.* Vol. 2. Tetra Tech, Inc., Bellevue, WA.

Tetra Tech. 1985. *Bioaccumulation monitoring guidance: Recommended analytical detection limits.* Vol. 3. Tetra Tech, Inc., Bellevue, WA.

Tetra Tech. 1986. *Bioaccumulation monitoring guidance: Analytical methods for U.S. EPA priority pollutants and 301(h) pesticides in tissues from estuarine and marine organisms.* Vol. 4. Tetra Tech, Inc., Bellevue, WA.

Tetra Tech. 1987. *Bioaccumulation monitoring guidance: Strategies for sample replication and compositing.* Vol. 5. Tetra Tech, Inc.

USEPA. 1982. *Method for use of caged mussels to monitor for bioaccumulation and selected biological responses of toxic substances in municipal wastewater discharges to marine waters.* Draft. U.S. Environmental Protection Agency, Environmental Monitoring Support Laboratory, Cincinnati, OH.

USEPA. 1985. *Bioaccumulation monitoring guidance: Selection of target species and review of available bioaccumulation data.* Vol. 2. EPA 403/9-86-006. Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1986. *Bioaccumulation monitoring guidance: Analytical methods for USEPA priority pollutants and 301(h) pesticides in tissues from estuarine and marine organisms.* Vol. 4. EPA 503/6-90-002. Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1987. *Quality assurance/quality control (QA/QC) for 301(h) monitoring programs: Guidance on field and laboratory methods.* EPA 430/9-86-004. Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1989. *Assessing human health risks from chemically contaminated fish and shellfish: A guidance manual.* U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1989. *Guidance manual: Bedded sediment bioaccumulation tests.* EPA/600/X-89/302. ERLN-N111. U.S. Environmental Protection Agency, Environmental Research Laboratory - Newport, OR.

USEPA. 1990. *Assessment and control of bioconcentratable contaminants in surface waters.* Draft report. U.S. Environmental Protection Agency, OWEP, Washington, DC.

USEPA. 1990. *Computerized Risk and Bioaccumulation System* (Version 1.0). ERLN-N137. U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, RI.

USEPA. 1991. *Technical support document for water quality-based toxics control.* EPA/505/2-90-001. U.S. Environmental Protection Agency, Washington, DC.

Young, D.R., A.J. Mearns, and R.W. Gosset. 1990. *Bioaccumulation and biomagnification of DDT and PCB residues in a benthic and a pelagic food web of Southern California.*

PATHOGENS

Andrews, W.H., and M.W. Presnell. 1972. Rapid recovery of *Escherichia coli* from estuarine water. *Appl. Microbiol.* 23:521.

APHA. 1989. American Public Health Association, American Water Works Association, American Water Pollution Control Federation. *Standard methods for the examination of water and wastewater.* 17th ed. American Public Health Association, Washington, DC.

Bisson, J.W., and V.J. Cabelli. 1979. Membrane filtration enumeration method for *Clostridium perfringens. Appl. Environ. Microbiol.* 37:55-66.

Bitton, G., B.N. Feldberg, and S.R. Farrah. 1979. Concentration of enteroviruses from seawater and tap water by organic flocculation using non-fat dry mile and casein water. *Air Soil Pollut.* 10:187.

Booz-Allen & Hamilton. 1983. *A background document on pathogenic organisms commonly found in municipal sludge.* Prepared for U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office, Cincinnati, OH.

Bordner, R.H., J.A Winter, and P.V Scarpino, eds. 1978. *Microbiological methods for monitoring the environment, water and waste.* EPA/600/8-78-017. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

Borrego, JJ., F. Arrabal, A. de Vicente, L.F. Gomez, and P. Romero. 1983. Study of microbial inactivation in the marine environment. *J. Water Pollut. Control Fed.* 55:297-302.

Brezenski, F.T., and J.A. Winter. 1979. Use of the delayed incubation membrane filter test for determining coliform/bacteria in sea water. *Water Res.* 3:583.

Buras, N. 1974. Recovery of viruses from waste-water and effluent by the direct inoculation method. *Water Res.* 8:19.

Cabelli, V.J. 1983. *Health effects criteria for marine waters.* EPA/600/1-80-0431, U.S. Environmental Protection Agency, Cincinnati, OH.

Cabelli, V.J., A.P. DuFour, M.A. Levin, L.J. McCabe, and P.W. Haberman. 1979. Relationshipi of microbial indicators to health effects at marine bathing beaches. *Am.J. Public Health* 69:690-696.

Cabelli, V.J., A.P. DuFour, L.J. McCabe, and M.A. Levin. 1982. Swimming-associated gastroenteritis and water quality. *Am. J. Epidemiol.* 115:606-61.

Cabelli, V.J., A.P. DuFour, L.J. McCabe, and M.A. Levin. 1983. A marine recreational water quality criterion consistent with indicator concepts and risk analysis. *J. Water Pollut. Control Fed.* 55:1306-1314.

CDC. 1979. Viral hepatitis outbreaks–Georgia, Alabama. Centers for Disease Control. *Morbid. Mortal. Weekly Rep.* 28:581.

Chang, S.L., G. Berg, K.A. Busch, R.E. Stevenson, N.A. Clarke, and P.W. Kabler. 1958. Application of the Most Probable Number method for estimating concentrations of animal viruses by tissue culture technique. *Virology* 6:27.

Clark, H.F., E.E. Geldreich, H.L. Jeter, and P.W Kabler. 1951. The membrane filter in sanitary bacteriology. *Pub. Health Rep.* 66:951.

Clark, J.A. 1969. The detection of various bacteria indicative of water pollution by a presence-absence (P-A) procedure. *Can. J. Microbiol.* 15:771.

Clark, J.A. 1980. The influence of increasing numbers of nonindicator organisms upon the detection of indicator organisms by the membrane filter and presence-absence tests. *Can. J. Microbiol.* 26:827.

Clark, J.A., and O.T. Vlassoff. 1973. Relationships among pollution indicator bacteria isolated from raw water and distribution systems by the presence-absence (P-A) test. *Health Lab. Sci.* 10:163.

Cliver, D.O. 1967. Detection of enteric viruses by concentration with polyethylene glycol. In *Transmission of viruses by the water route*, ed. G. Berg. Interscience Publ., New York, NY.

Cooney, M.K. 1973. Relative efficiency of cell cultures for detection of viruses. *Health Lab. Sci.* 4:295.

Cowles, P.B. 1939. A modified fermentation tube. *J. Bacteriol.* 38:677.

Dalla Vallee, J.M. 1941. Notes on the most probable number index as used in bacteriology. *Pub. Health Rep.* 56:229.

Dobberkau, H.J., R. Walter, and S. Rudiger. 1981. Methods for virus concentration from water. In *Viruses and wastewater treatment*, ed. M. Goddard and M. Butler. Pergamon Press, New York, NY.

DuFour, A.P., E.R. Strickland, and V.J. Cabelli. 1981. Membrane filter method for enumerating *Escherichia coli. Appl. Environ. Microbiol.* 41:1152-1158.

Dutka, B.D. 1981. *Membrane filtration applications, techniques and problems.* Marcel Dekker, Inc., New York, NY.

Emerson, D.J., and V.J. Cabelli. 1982. Extraction of *Clostridium perfingens* spores from bottom sediment samplers. *Appl. Environ. Microbiol.* 44:1152-1158.

Ericksen, T.H., C. Thomas, and A. Dufour. 1983. *Comparison of two selective membrane filter methods for enumerating fecal streptococci in freshwater samples.* Abs. Annual Meeting, American Soc. Microbiology, p. 279.

Evans, T.M., C.E. Warvick, R.J. Seidler, and M.W. LeChevallier. 1981. Failure of the most-probable number techniques to detect coliforms in drinking water and raw water supplies. *Appl. Environ. Microbiol.* 41:130.

Farrah, S.R. 1982. Isolatin of viruses associated with sludge particles. In *Methods in environmental virology*, ed. C.P. Gerba and S.M. Goyal. Marcel Dekker, Inc., New York, NY.

Feingold, A.O. 1973. Hepatitis from eating steamed clams. *J. Am. Med. Assoc.* 225:526-527.

Ferraro, S.P., F.A. Cole, W.A. DeBen, and R.C. Swartz. 1989. Power-cost efficiency of eight macrobenthic sampling schemes in Puget Sound, Washington, USA. *Can. J. Fish. Aquat. Sci.* 46:2157-2165.

Fifield, C.W., and C.P. Schaufus. 1958. Improved membrane filter medium for the detection of coliform-organisms. *J. Amer. Water Works Assoc.* 50:193.

Gameson, A.L.N. 1983. Investigation of sewage discharges to some British coastal waters. Water Resources Centre Technical Report, TR 193. Bucks, United Kingdom.
Geldreich, E.E., P.W. Kabler, H.L. Jeter, and H.F. Clark. 1955. A delayed incubation membrane filter test for coliform bacteria in water. *Amer. J. Pub. Health* 45:1462.

Geldreich, E.E., H.L. Jeter, and J.A. Winter. 1967. Technical considerations in applying the membrane filter procedure. *Health Lab. Sci.* 4:113.

Gerba, C.P., and S.M. Goyal. 1982. *Methods in environmental virology.* Marcel Dekker, Inc., New York, NY.

Gerhards, P., ed. 1981. *Manual of methods for general bacteriology.* American Soc. Microbiology, Washington, DC.

Greenberg, A.E., and D.A. Hunt, eds. 1985. *Laboratory procedures for the examination of seawater and shellfish.* 5th ed. American Public Health Association, Washington, DC.

Halvorson, H.O., and N.R. Ziegler. 1933-35. Application of statistics to problems in bacteriology. *J. Bacteriol.* 24:101; 26:4331, 559, 29:609.

Hardy, J.T. 1982. The sea surface microlayer: Biology, chemistry, and anthropogenic enrichment. *Prog. Oceanogr.* 11:307-328.

Homma, A., M.D. Sobsey, C.Wallis, and J.L. Melnick. 1973. Virus concentration from sewage. *Water Res.* 7:945.

Hsiung, G.D. 1973. *Diagnostic virology.* Revised ed. Yale Univ. Press, New Haven, CT.

Inhorn, S.L., ed. 1977. *Quality assurance practices for health laboratories.* American Public Health Assoc., Washington, DC.

Jacobs, N.J., W.L. Zeigler, F.C. Reed, T.A. Stukel, and E.W. Rice. 1986. Comparison of membrane filter, multiple-fermentation-tube, and presence-absence techniques for detecting total coliforms in small community water systems. *Appl. Environ. Microbiol.* 51:1007.

Kaplan, J.E., R.A. Goodman, L.B. Schonberger, E.C. Lippy, and G.W. Gary. 1982. Gastroenteritis due to Norwalk virus: An outbreak association with municipal water system. *J. Infect. Dis.* 146:190-197.

Kabler, P.W. 1954. Water examinations by membrane filter and MPN procedures. *Amer. J. Pub. Health* 44:379.

Kelly, S., and W.W. Sanderson. 1962. Comparison of various tissue cultures for the isolation of enteroviruses. *Amer. J. Pub. Health* 52:455.

Lee, L.H., C.A. Phillips, M.A. South, J.L. Melnick, and M.D. Yow. 1965. Enteric virus isolations in different cell cultures. *Bull. World Health Org.* 32:657.

Lennette, E.H., A. Balows, W.J. Hausler, Jr., and H.J. Shadomy, eds. 1985. *Manual of clinical microbiology.* 4th ed. American Soc. for Microbiology, Washington, DC.

Levin, M.A., J.R. Fischer, and V.J. Cabelli. 1975. Membrane filter technique for enumeration of enterococci in marine waters. *Appl. Microbiol.* 30:66.

Lin, S. 1973. Evaluation of coliform test for chlorinated secondary effluents. *J. Water Pollut. Control Fed.* 45:498.

Lin, S.D. 1976. Evaluation of Millipore HA and HC membrane filters for the enumeration of indicator bacteria. *Appl. Environ. Microbiol.* 32:300.

Lund, E., and C.E. Hedstrom. 1969. A study on sampling and isolation methods for the detection of virus in sewage. *Water Res.* 3:823.

Lydholm, B., and A.L. Nielsen. 1979. Methods for detection of virus in wastewater applied to samples from small scale treatment systems. *Water Res.* 14:169.

McCarthy, J.A., H.A. Thomas, and J.E. Delaney. 1958. Evaluation of reliability of coliform density test. *Amer. J. Pub. Health* 48:12.

McCarthy, J.A., and J.E. Delaney. 1958. Membrane filter media studies. *Water Sewage Works* 105:292.

McCarthy, J.A., J.E. Delaney, and R.J. Grasso. 1961. Measuring coliforms in water. *Water Sewage Works* 108:238.

McCarthy, J.A., and J.E. Delaney. 1965. *Methods for measurig the coliform content of water.* Sec. III. Delayed holding procedures for coliform bacteria. PHS Res. Grant WP 00202 NIH Rep.

McCrady, M.H. 1915. The numerical interpretation of fermentation tube results. *J. Infect. Dis.* 12:183.

Morris, R., and W.M. Waite.1980. Evaluation of procedures for recovery of viruses from water - II detection systems. *Water Res.* 14:795.

NOAA. 1988. *National Marine Pollution Program federal plan for ocean pollution research, development and monitoring.* U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Washington, DC.

Ohara, H., H. Naruto, W. Watanabe, and I. Ebisawa. 1983. An outbreak of Hepatitis A caused by consumption of raw oysters. *J. Hyg. Camb.* 91:163-165.

Olson, B.H. 1978. Enhanced accuracy of coliform testing in seawater by a modification of the most-probable number method. *Appl. Microbiol.* 36:438.

OTA. 1987. *Wastes in marine environments.* U.S. Congress, Office of Technology Assessment. OTA 0-334.

Panezai, A.K., T.J. Macklin, and H.G. Coles. 1965. Coli-aerogenes and *Escherichia coli* counts on water samples by means of transported membranes. *Proc. Soc. Water Treat. Exam.* 14:179.

Payment, P., C.P. Gerba, C. Wallis and J.L. Melnick. 1976. Methods for concentrating viruses from large volumes of estuarine water on plated membranes. *Water Res.* 10:893.

Pederson, D.C. 1980. *Density levels of pathogenic organisms in municipal wastewater sludges: A literature review.* Prepared by Camp Dresser and McKee, Inc. for U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.

Ramia, S., and S.A. Sattar. 1979. Second-step concentration of viruses in drinking and surface waters using polyethylene glycol hydroextraction. *Can. J. Microbiol.* 25:587.

Rao, V.C., U. Chandorkar, N.U. Rao, P. Kumaran, and S.B. Lakhe. 1972. A simple method for concentrating and detecting viruses in wastewater. *Water Res.* 6:1565.

Rehm, R., S. Duletsky, J. Pierce, and R. Sommer. 1983. *Contaminants of concern in sewage sludge.* Draft prepared for Office of Program Policy and Evaluation, U.S. Environmental Protection Agency, Washington, DC.

Rovozzo, G.C., and C.N. Burke. 1973. *A manual of basic virological techniques.* Prentice-Hall, Englewood Cliffs, NJ.

Schmidt, N.J., H.H. Ho, J.L. Riggs, and E.H. Lennette. 1978. Comparative sensitivity of various cell culture systems for isolation of viruses from wastewater and fecal samples. *Appl. Environ. Microbiol.* 36:480.

Seidler, R.J., T.M. Evans, J.R. Kaufman, C.E. Warvick, and M.W. LeChevallier. 1981. Limitations of standard coliform enumeration techniques. *J. Amer. Water Works Assoc.* 73:538.

Selna, M.W., and R.P. Miele. 1977. Virus sampling in wastewater-field experiences. *J. Environ. Eng. Div., Proc. Amer. Soc. Civil Eng.* 103:693.

Shuval, H.I., S. Cymbalista, B. Fattal, and N. Goldblum. 1967. Concentration of enteric viruses in water by hydro-extraction and two-phase separation. *Transmission of viruses by the water route*, ed. G. Berg. Interscience Publ., New York, NY.

Shuval, H.I., B. Fattal, S. Cymbalista, and N. Goldblum. 1969. The phase-separation method for the concentration and detection of viruses in water. *Water Res.* 3:225.

Slanetz, L.W., and C.H. Bartley. 1957. Numbers of enterococci water, sewage and feces determined by the membrane filter technique with an improved medium. *J. Bacteriol.* 74:591.

Sobsey, M.D. 1976. Methods for detecting enteric viruses in water and wastewater. In *Viruses in water*, ed. G. Berg, H.L. Bodily, E.H. Lennette, J.L. Melnick and T.G. Metcalf, American Public Health Assoc., Washington, DC.

Sobsey, M.D. 1976. Field monitoring techniques and data analysis. In *Virus aspects of applying municipal waste to land*, ed. L.B. Baldwin, J.M. Davidson, and J.F. Gerber. Univ. Florida, Gainesville, FL.

Sobsey, M.D. 1982. Quality of currently available methodology for monitoring viruses in the environment. *Environ. Internat.* 7:39.

Sobsey M.D., C.P. Gerba, C. Wallis, and J.L. Melnick. 1977. Concentration of enteroviruses from large volumes of turbid estuary water. *Can. J. Microbiol.* 23:770.

St. John, E.W., J.R. Matches, and M.M. Wekell. 1982. Use of iron milk medium for enumeration of *Clostridium perfrigens. J. Assoc. Off. Anal. Chem.* 65:1129-1133.

Strandridge, J.H., and J.J Delfino. 1981. A-1 Medium: Alternative techniques for fecal coliform organism enumeration in chlorinated wastewaters. *Appl. Environ. Microbiol.* 42:918.

Thomas, H.A., Jr. 1942. Bacterial densities from fermentation tube test. *J. Amer. Water Works Assoc.* 34:572.

Taylor, R.H., R.H. Bordner, and P.V. Scarpino. 1973. Delayed incubation membrane-filter test for fecal coliforms. *Appl. Microbiol.* 25:363.

Thomas, H.A., and R.L. Woodward. 1956. Use of molecular filter membranes for water potability control. *J. Amer. Water Works Assoc.* 48:1391.

USEPA. 1978. *Microbiological methods for monitoring the environment.* EPA 600/8-78-017. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory.

USEPA. 1985. *Test methods for* Escherichia coli *and enterococci in water by the membrane filter procedure.* U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

USEPA. 1986. *Ambient water quality criteria for bacteria - 1986.* EPA 440/5-84-002. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1986-1991. *Recommended protocols for measuring selected environmental variables in Puget Sound.* Looseleaf. U.S. Environmental Protection Agency, Region 10, Puget Sound Estuary Program, Seattle, WA.

USEPA. 1988. *Water quality standards criteria summaries: A compilation of state/federal criteria - Bacterial.* U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC.

Wallis, C., and J.L. Melnick. 1967. Virus concentration on aluminum and calcium salts. *Amer. J. Epidemiol.* 85-459.

Wallis, C., and J.L. Melnick. 1967. Concentration of viruses on aluminum hydroxide precipitates. In *Transmission of viruses by the water route,* ed. G. Berg. Interscience Publ., New York, NY.

Wellings, F.M., A.L. Lewis, and C.W. Mountain. 1976. Viral concentration techniques for field sample analysis. In *Virus aspects of applying municipal waste to land,* ed. L.B.Baldwin, J.M. Davidson, and J.F. Gerber. Univ. Florida, Gainesville, FL.

Weiss, J.E., and C.A. Hunter. 1939. Simplified bacteriological examination of water. *J. Amer. Water Works Assoc.* 31:689.

EFFLUENT CHARACTERIZATION

Adelman, I.R., L.L. Smith Jr., and G.D. Siesennop. 1976. Acute toxicity of sodium chloride, pentachlorophenol, guthion, and hexavalent chromium to fathead minnows (*Pimephales promelas*) and goldfish (*Carassius auratus*). *J. Fish. Res. Board Can.* 33:203-208.

APHA. 1989. American Public Health Association, American Water Work Association, Water Pollution Control Federation. *Standard methods for the examination of water and wastewater.* 17th ed. American Public Health Association, Washington, DC.

Bergman, H., R. Kimerle, and A.W. Maki, eds. 1985. *Environmental hazard assessment of effluents.* Pergamon Press, Inc. Elmsford, NY.

Bowman, M.C., W.L. Oller, T. Cairns, A.B. Gosnell, and K.H. Oliver. 1981. Stressed bioassay systems for rapid screening of pesticide residues. Part I: Evaluation of bioassay systems. *Arch. Environ. Contam. Toxicol.* 10:9-24.

Dowden, B.F., and H.J. Bennett. 1965. Toxicity of selected chemicals to certain animals. *J. Water Pollut. Control Fed.* 37(9):1308-1316.

Kimerle, R., W. Adams, and D. Grothe. 1985, Tiered assessment of effluents. In *Environmental hazard assessment of effluents*, eds. H. Bergman, R. Kimerle, and A. Maki.

Kimerle, R., A. Werner, and W. Adams. 1983. Aquatic hazard evaluation, principles applied to the development of water quality criteria. In *Aquatic toxicology and hazard assessment* (7th symposium), eds. R. Cardwell and R. Purdy.

Magnuson, V.R., D.K. Harriss, M.S. Sun, D.K. Taylor, and G.E. Glass. 1979. *Relationships of activities of metal-ligand species of aquatic toxicity.* ACS symposium series, no. 93. Chemical modeling in aqueous systems, ed. E.A. Henne, pp. 635-656.

OSHA. 1976. *OSHA safety and health standards, general industry.* OSHA 2206 (revised). 29 CFR 1910. Occupational Safety and Health Administration, Washington, DC.

Patrick, R., J. Cairns, Jr., and A. Scheier. 1968. The relative sensitivity of diatoms, snails, and fish to twenty common constituents of industrial wastes. *Prog. Fish-Cult.*:137-140.

Poirier, S.H., M.L. Knuth, C.D. Anderson-Buchou, L.T. Brooke, A.R. Lima, and P.J. Shubat. 1986. Comparative toxicity of methanol and N,N-dimethylformamide to freshwater fish and invertebrates. *Bull. Environ. Contam. Toxicol.* 37(4):615-621.

Randall, T.L., and P.V. Knopp. 1980. Detoxification of specific organic substances by wet oxidation. J. Water Pollut. Control Fed. 52(8): 217-2130.

Schimmel, S.C., G.E. Morrison, and M.A. Heber. 1989. Marine complex effluent toxicity program: Test sensitivity, repeatability, and relevance to receiving water toxicity. *Env. Tox. and Chem.* 8:739-746.

Stumm, W., and J.J. Morgan. 1981. *Aquatic chemistry - An introduction emphasizing chemical equilibria in natural waters.* John Wiley and Sons, Inc., New York, NY.

U.S. Department of Health, Education and Welfare. 1977. *Carcinogens - working with carcinogens.* Publication no. 77-206. Public Health Service, Center for Disease Control, National Institute of Occupational Safety and Health.

USEPA. 1982. *Handbook for sampling and sample preservation of water and wastewater.* EPA 600/4-82-019. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

USEPA. 1982. *Test methods - Technical additions to methods for chemical analysis of water and wastes.* EPA 600/4-82-055. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH. December.

USEPA. 1982. *Water quality assessment: A screening procedure toxic and conventional pollutants.* Parts 1 and 2. EPA 600/6-82-004. U.S. Environmental Protection Agency, Office of Research and Development, Athens, GA.

USEPA. 1983. *The treatability manual.* Vol. IV. EPA 600/2-82-001. U.S. Environmental Protection Agency, Office of Research and Development. GPO, Washington, DC.

USEPA. 1984. *Effluent and ambient toxicity testing and instream community response on the Ottawa River, Lima, Ohio.* EPA 600/2-84-080. Permits Division, Washington, DC, U.S. Environmental Protection Agency, Office of Research and Development, Duluth, MN.

USEPA. 1984. CETIS: *Complex Effluent Toxicity Information System. Data encoding guidelines and procedures.* EPA 600/8-84-029. U.S. Environmental Protection Agency, Office of Research and Development, Duluth, MN.

USEPA. 1984. CETIS: *Complex Effluent Toxicity Information System. CETIS retrieval system user's manual.* EPA 600/8-84-030. U.S. Environmental Protection Agency, Office of Research and Development, Duluth, MN.

USEPA. 1984. Development of water quality based permit limitations for toxic pollutants; National policy. U.S. Environmental Protection Agency. *Fed. Regist.*, March 9, 1984, 49(48).

USEPA. 1984. *Technical guidance manual for performing wasteload allocations, Book III estuaries.* U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC.

USEPA. 1985. *Methods for measuring the acute toxicity of effluents freshwater and marine organisms.* EPA 600/4-85-013. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

USEPA. 1985. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms.* EPA 600/4-85-014. U.S. Environmental Protection Agency, Cincinnati, OH.

USEPA. 1986-1988. *Quality criteria for water.* EPA 440/5-86-001. U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC.

USEPA. 1988. *Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures.* Draft EPA research series report. EPA 600/3-88-034. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

USEPA. 1988. *Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures.* Draft EPA research series report. EPA 600/3-88-035. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

USEPA. 1988. *Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures.* Draft phase III toxicity series report. EPA/600/3-88-036. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

USEPA. 1988. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms.* EPA 600/4-87-02. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.

USEPA. 1988. *Draft generalized methodology for conducting industrial toxicity reduction evaluations (TREs).* Draft EPA Research Series Report. U.S. Environmental Protection Agency, Water Engineering Research Laboratory, Cincinnati, OH.

USEPA. 1989. T*oxicity reduction evaluation protocol for municipal wastewater treatment plants.* EPA 600/2-88-062. U.S. Environmental Protection Agency, Water Engineering Research Laboratory, Cincinnati, OH.

USEPA. 1989. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms.* EPA 600/4-89-001. U.S. Environmental Protection Agency, Water Engineering Research Laboratory, Cincinnati, OH.

USEPA. 1989. *Biomonitoring for control of toxicity in effluent discharges to the marine environment.* EPA 625/8-89-015. U.S. Environmental Protection Agency, Office of Research and Development, Center for Environmental Research Information.

USEPA. 1990. *Permit writer's guide for marine and estuarine discharges.* Draft. U.S. Environmental Protection Agency, Office of Water Enforcement and Permits, Washington, DC.

USEPA. 1990. *Assessment and control of bioconcentratable contaminants in surface waters.* Draft. U.S. Environmental Protection Agency, Office of Water Enforcement and Permits, Washington, DC.

USEPA. 1991. *Technical support document for water quality-based toxics control.* EPA 505/2-90-001. U.S. Environmental Protection Agency, Office of Water Enforcement and Permits, Office of Water Regulations and Standards, Washington, DC.

Walters, C.I., and C.W. Jameson. 1984. *Health and safety for toxicity testing.* Butterworth Publ., Woburn, MA.

MESOCOSMS AND MICROCOSMS

Davey, E.W., K.T. Perez, A.E. Soper, N.F. Lackie, G.E. Morrison, R.L. Johnson, and J. F. Heltsche. In press. *Significance of the surface microlayer to the environmental fate of di(2-ethylhexyl) phthalate predicted from marine microcosms.* U.S. Environmental Protection Agency, Environmental Research Laboratory, Ecosystems Effects Branch, Narragansett, RI.

Donaghay, P.L. 1984. Utility of mesocosms to assess marine pollution. In *Concepts in marine pollution measurements*, ed. H.H. White, pp. 589-620. Maryland Sea Grant College, College Park, MD.

Dwyer, R.L., and K.T. Perez. 1983. An experimental examination of ecosystem linearization. *The American Naturalist* 121 (3):305-323.

Grassle, J.P., and J.F. Grassle. 1984. The utility of studying the effects of pollutants on single species populations in benthos of mesocosms and coastal ecosystems. In *Concepts in marine pollution measurements*, ed. H.H. White, pp. 621-642. Maryland Sea Grant College, College Park, MD.

Grice, G.W. 1984. Use of enclosures in studying stress on plankton communities. In *Concepts in marine pollution measurements*, ed. H.H. White, pp. 563-575. Maryland Sea Grant College, College Park, MD.

Leffler, J.W. 1984. The use of self-selected, generic aquatic microcosms for pollution effects assessment. In *Concepts in marine pollution measurements*, ed. H. White, pp. 139-158. Maryland Sea Grant College, College Park, MD.

Oviatt, C.A. 1984. Ecology as an experimental science and management tool. In *Concepts in marine pollution measurements*, ed. H.H. White, pp. 539-548 Maryland Sea Grant College, College Park, MD.

Perez, K.T., E.W. Davey, N.F. Lackie, G.E. Morrison, P.G. Murphy, A.E. Soper, and D.L. Winslow. 1984. *Environmental assessment of phthalate ester, di(2-ethylhexyl) phthalate (DEHP), derived from a marine microcosm.* Special Technical Publication 802. American Society for Testing and Materials (ASTM), Philadelphia, PA.

Perez, K.T., and G.E. Morrison. 1985. Environmental assessments from simple test systems and a microcosm: Comparisons of monetary costs. In *Multispecies toxicity testing*, ed. J. Cairns, pp. 89-95.

Perez, K.T., E.W. Davey, G.E. Morrison, J.A. Cardin, N.F. Lackie, A.E. Soper, R.J. Blasco, C. Bearce, R.L. Johnson, and S. Marino. 1989. *Influence of organic matter and industrial contaminants in sewage effluent on marine ecosystems.* ERLN Publication. U.S. Environmental Protection Agency, Environmental Research Laboratory, Ecosystems Effects Branch, Narragansett, RI.

Perez, K.T., E.W. Davey, J. Heltsche, J.A. Cardin, N.F. Lackie, R.L. Johnson, R.J. Blasco, A.E. Soper, and E. Read. 1990. *Recovery of Narragansett Bay, RI: A feasibility study.* ERLN Contribution No. 1148. U.S. Environmental Protection Agency, Environmental Research Laboratory, Ecosystems Effects Branch, Narragansett, RI.

Perez, K.T., G.E. Morrison, E.W. Davey, N.F. Lackie, A.E. Soper, R.J. Blasco, D.L. Winslow, R.L. Johnson, P.G. Murphy, J.F. Heltsche. In press. *Influence of size on the fate and ecological effects of the pesticide kepone in a physical simulation model.* U.S. Environmental Protection Agency, Environmental Research Laboratory, Ecosystems Effects Branch, Narragansett, RI.

Pilson, M.E.Q. 1984. Should we know the fates of pollutants. In *Concepts in marine pollution measurements,* ed. H.H. White, pp. 575-588. Maryland Sea Grant College, College Park, MD.

Pontasch, K.W., B.R. Niederlehner, and J. Cairns, Jr. 1989. Comparisons of single-species microcosm and field responses to a complex effluent. *Environ. Tox. and Chem.* 8:521-532.

Pritchard, P.H., and A.W. Bourquin. 1984. A perspective on the role of microcosms in environmental fates and effects assessments. In *Concepts in marine pollution measurements*, ed. H.H. White, pp. 117-138. Maryland Sea Grant College, College Park, MD.

Santschi, P.H., U. Nyffeler, R. Anderson, and S. Schiff. 1984. The enclosure as a tool for the assessment of transport and effects of pollutants in lakes. In *Concepts in marine pollution measurements,* ed. H.H. White, pp. 549-562. Maryland Sea Grant College, College Park, MD.

Taub, F.B. 1984. Introduction to laboratory microcosms. In *Concepts in marine pollution measurements*, ed. H.H. White, pp. 113-116. Maryland Sea Grant College, College Park, MD.

Taub, F.B. 1984. Measurement of pollution in standardized aquatic microcosms. In *Concepts in marine pollution measurements*, ed. H.H. White, pp. 159-192. Maryland Sea Grant College, College Park, MD.

USEPA. 1983. *Project summary: Experimental marine microcosm test protocol and support document.* EPA-600/S3-83-055. U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, RI.

USEPA. 1987. Site-specific aquatic microcosm test. *Fed. Regist.* 52(187): 36352-36360.

USEPA. 1990. *Experimental marine microcosm test protocol and support document.* Revised. U.S. Environmental Protection Agency, Environmental Research Laboratory, Ecosystems Effects Branch Narragansett, RI.

APPENDIX B:

OCEAN DISCHARGE CRITERIA
PUBLISHED AT FR Vol. 45, No. 194, 65942-65954
OCTOBER 3, 1980

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 125

[FRL 1609-1]

Ocean Discharge Criteria

AGENCY: Environmental Protection Agency.

ACTION: Final rule.

SUMMARY: EPA is promulgating final guidelines under section 403(c) of the Clean Water Act. These guidelines will be applied in issuing and revising National Pollutant Discharge Elimination System Permits for discharges into the territorial seas, the contiguous zone and the oceans.

DATES: These guidelines become effective on November 3, 1980.

FOR FURTHER INFORMATION CONTACT: Kenneth Farber, Office of Water Regulations and Standards (WH-586), Environmental Protection Agency, 401 M Street, SW, Washington, D.C. 20460, 202-472-5746.

SUPPLEMENTARY INFORMATION:

I. Background

EPA is today promulgating revised guidelines for determining the degradation of the territorial seas, the contiguous zone and the oceans. Pursuant to section 403(a) of the Clean Water Act, no National Pollutant Discharge Elimination System ("NPDES") permit for discharges into these marine waters may be issued when these guidelines are in effect except in compliance with the guidelines.

These guidelines are issued pursuant to section 403(c)(1) which provides that:

> The Administrator shall, within one hundred and eighty days after enactment of this Act (and from time to time thereafter), promulgate guidelines for determining the degradation of the waters of the territorial seas, the contiguous zone, and the ocean, which shall include:
>
> (A) the effect of disposal of pollutants on human health or welfare, including but not limited to plankton, fish, shellfish, wildlife, shorelines, and beaches.
>
> (B) the effect of disposal of pollutants on marine life, including the transfer, concentration, and dispersal of pollutants or their byproducts through biological, physical, and chemical processes; changes in marine ecosystem diversity, productivity, and stability; and species and community population changes;
>
> (C) the effect of disposal of pollutants on esthetic, recreation, and economic values;
>
> (D) the persistence and permanence of the effects of disposal of pollutants;
>
> (E) the effect of the disposal at varying rates, of particular volumes and concentrations of pollutants;
>
> (F) other possible locations and methods of disposal or recycling of pollutants including land-based alternatives; and
>
> (G) the effect on alternate uses of the oceans, such as mineral exploitation and scientific study.

On October 15, 1973, EPA promulgated combined regulations implementing section 102(a) of the Marine Protection, Research, and Sanctuaries Act and section 403(c) of the Clean Water Act. The primary focus of these regulations was on the ocean disposal of waste material, including sewage sludges, liquid and solid industrial wastes and dredged materials, by dumping from moving vessels.

In practice, these regulations proved unworkable in many respects as section 403 ocean discharge criteria. At the same time, operating experience demonstrated that the ocean dumping regulations themselves required revision. EPA therefore determined that both the ocean dumping regulations and the ocean discharged criteria should be revised and published as separate regulations. All reference to section 403(c) guidelines was deleted from the revised ocean dumping regulations which were promulgated on January 11, 1977 (42 FR 2468). However, the Agency encountered substantial difficulty in developing revised ocean discharge guidelines, and, until recently, there have been no published national guidelines in place. Since withdrawal of the original guidelines, permit writers have been implementing section 403 on a case-by-case basis.

On June 21, 1979, the Pacific Legal Foundation filed suit in United States District Court for the Eastern District of California, seeking, among other things, that EPA promulgate revised section 403(c) guidelines, *Pacific Legal Foundation* v. *Costle*, Civ. No. S-79-429-PCW. The National Wildlife Federation intervened in that lawsuit. On October 31, 1979, the Court ordered EPA both to promulgate these guidelines and to publish interim guidelines stating Agency policy in reviewing, issuing, or denying NPDES permits under section 403, pending promulgation of the final guidelines. The interim guidelines were published in the **Federal Register** on November 15, 1979, 44 FR 65751, and they will be superseded by the final guidelines published today.

The Agency then published proposed ocean discharge criteria in the **Federal Register** (45 FR 9548) on February 12, 1980, held an oral hearing on the proposal on March 21, 1980, and provided a comment period for submission of written comments which was to end on March 28, 1980. At the request of various interested groups, the comment period was extended for 30 days. Based on the extended comment period and on the large volume of comments received, the Agency moved the court to extend the final promulgation date by 120 days beyond the July 30 deadline. The court extended the final deadline until September 30, 1980.

II. Development of the 403 Guidelines

1. Synopsis of the Guidelines

Section 403 is intended to prevent unreasonable degradation of the marine environment and to authorize imposition of effluent limitations, including a prohibition of discharge, if necessary, to ensure this goal. These guidelines were developed to satisfy this intent. They provide flexibility to permit writers to tailor application requirements, effluent limitations, and reporting requirements to the specific circumstances of each discharger's situation, while ensuring consistency and certainty by imposing minimum requirements, in situations where the long-term impact of a discharge is not fully understood.

Under these guidelines, no NPDES permit may be issued which authorizes a discharge of pollutants that will cause unreasonable degradation of the marine environment. Prior to permit issuance, the director, defined as either the Regional Administrator or the State Director where there is an approved State program, or an authorized representative, is required to evaluate whether a proposed discharge will cause such degradation. In making this determination, the director is to consider the factors specified in § 125.122 (a) and (b).

In cases where sufficient information is available for the director to make a reasonable determination whether unreasonable degradation of the marine environment will occur, the director is governed by § 125.123 (a) and (b) of the regulations. Discharges which will cause unreasonable degradation will be prohibited; other discharges may be permitted under conditions necessary to ensure that such degradation will not occur.

In those cases where the director is unable to determine whether unreasonable degradation will occur, § 125.123(c) governs. No discharge in this situation is allowed unless the director can reasonably determine that: (1) the discharge will not cause irreparable harm to the marine environment while further evaluation is undertaken; (2) there are no reasonable

alternatives to the discharge; and (3) the discharge will comply with certain mandatory permit conditions, including a bioassay-based discharge limitation and monitoring requirements. These permit conditions will assist in determining whether and to what extent further limitations are necessary to ensure that the discharge does not cause unreasonable degradation. If further limitations are necessary, § 125.123(d)(4) provides that the permit must be then modified to include these additional limitations or else revoked.

These guidelines encourage the use of available information in addition to any supplied by the permit applicant. Thus, the director may make determination based on information such as that contained in any relevant environmental impact statement, section 301(h) or other variance applications, existing technical and environmental field studies, or EPA industrial and municipal waste surveys.

2. Relationship Between the Statute and the Guidelines

(a) *Section 403(c)(1)*—Section 403(c)(1) specifies seven factors which are to be included in guidelines for determining the degradation of marine waters. These factors form the basis for the determinations which must be made pursuant to these guidelines.

Most of the statutory factors, including 403(c)(1)(A), (B), (C), (D), (E), and a portion of (G), involve consideration of the biological effects of the discharge of pollutants. These factors, either directly or indirectly, must be evaluated by the director in determining whether a discharge will cause unreasonable degradation of the marine environment. Section 125.122(a) requires that the director assess such variables as the location of the discharge, including the composition of the biological community and existence of special aquatic sites, such as marine sanctuaries; the nature of the pollutants which are to be discharged, including their quantities, composition, potential for bioaccumulation, persistence and their transport in the environment, and the effect on human health. This assessment should adequately address the statutory factors relating to biological effects of the discharge.

Section 403(c)(1)(C) also involves consideration of economic and social impacts of the discharge, as does section 403(c)(1)(G). The guidelines address these factors in assessing whether a discharge will cause unreasonable degradation of the marine environment. Section 122.121(f) defines "unreasonable degradation of the marine environment" to include, among other things, "loss of esthetic, recreational or economic values which are unreasonable in relation to the benefit derived from the discharge." Thus, even where the director has determined that there are no significant changes in ecosystem diversity, productivity and stability, and there is no threat to human health, he may conclude that a discharge may not be authorized if the adverse impact on such activities as fishing, recreation, and/or other economic or social benefits is unreasonable in relation to benefits, such as oil and gas production, derived from the discharge.

Section 403(c)(1)(F) involves consideration of other possible locations and disposal methods for pollutants. Although EPA has considered this factor in developing these guidelines, the director is not required to assess alternatives in all cases. Under section 125.123(c)(2) the director must assess the availability of alternatives, including land-based alternatives, only in those cases where he cannot determine that a discharge will not cause unreasonable degradation of the marine environment.

Additionally, the guidelines establish a presumption that discharges in compliance with sections 301(h), 316(a), 301(g) and State water quality standards under section 303 will not cause unreasonable degradation. Although the director may, on the basis of the factors specified in § 125.122(a), conclude that additional permit limitations are in fact necessary even though the requirements of these other sections have been met, the similarity between the objectives and requirements of these provisions and those of section 403 warrants a presumption that discharges in compliance with these sections also satisfy section 403. Also, even though the regulations provide that a successful section 316(a) demonstration creates only a rebuttable presumption that section 403 has been satisfied, the provisions of section 316(c) may in some cases preclude the imposition of more stringent limitations under section 403.

POTWs obtaining section 301(h) variances are entitled to a presumption that their entire discharge is in compliance with section 403. However, the presumption applies only to the thermal component of a discharge subject to a 316(a) variance or to those specific non-conventional pollutants subject to a section 301(g) variance or to pollutants specifically limited by criteria in State water quality standards. Each of those provisions, like section 403, is geared toward assessing the environmental impact of a discharge. In order for a point source to receive a section 301(g), 301(h) or 316(a) variance, an evaluation of the biological and environmental effect of the discharge is required. Indeed, the statutory factors specified in these sections are similar to those contained in section 403(c). Similarly, State water quality standards established pursuant to section 303 of the Act are designed to preserve the quality of waters under State jurisdiction, including the territorial seas, and compliance with these standards should insure protection of the uses for which the waters are designated with respect to pollutants for which standards have been established.

(b) *Section 403(c)(2)*—Section 403(c)(2) states that:

> Where insufficient information exists on any proposed discharge to make a reasonable judgment on any of the guidelines established pursuant to this section no permit shall be issued under section 402 of this Act.

This section is the basis for two central elements of these requlations. First, the guidelines require that the director make potentially complex factual determinations on the basis of information which, in many cases, may be conflicting and in dispute. Section 403(c)(2) provides that the standard on which the director is to make these judgments is one of "reasonableness." In assessing the information in the administrative record, the director may authorize the discharge of pollutants if he is able to make a "reasonable judgment" about the determinations specified in the guidelines. Although these issues may involve scientific matters, the director is not bound by the same burden of proof which a scientist might require to reach a conclusion. The administrative process and the burden of proof in making these determinations are discussed below.

Second, the regulation provides, as required by section 403(c)(2), that the director may not authorize the discharge of pollutants if there is insufficient information to make these judgments. The regulation does not, however, require that there be complete knowledge of the impact of a discharge prior to permit issuance. Section 125.123(c) provides that a permit may be issued if the director has sufficient information to reasonably conclude, among other things, that the discharge will not cause irreparable harm to the environment while additional information is collected. The provision implements Congress' intent that "discharges permitted today will not irreversibly modify the oceans for future uses." S. Rep. No. 92–414, 92nd Cong., 1st Sess. at 75 (1971). It should insure adequate protection of the environment at all times while allowing the director to issue NPDES permits where existing

data may not be adequate to assess the long term impact of a discharge.

3. The Role of Section 403(c) Guidelines in NPDES Permit Issuance

These guidelines will be used to develop NPDES permits for the discharge of pollutants into the territorial seas, the contiguous zone and the oceans. Application of the guidelines will aid in protecting marine resources and their uses from the impact of pollution and in preventing unreasonable degradation of the marine environment.

Although sometimes described here as "guidelines" or "criteria", these promulgated regulations establish minimum requirements on discharges to protect the receiving waters. These guidelines will be used in evaluating applications for new, modified or renewed permits as they are submitted.

These guidelines apply in addition to other applicable provisions of the Clean Water Act. Permittees subject to section 403 must still comply with all other requirements of the Act, including applicable technology-based requirements specified by sections 301, 304 or 306 and water-quality based limitations specified by sections 303 or 307. Permittees may in certain circumstances be subject to the provisions of section 311 as well.

Section 403 applies to all discharges seaward of the inner boundary of the territorial seas. This boundary is defined by section 502(a) of the Act to be the—

> belt of the seas measured from the line of ordinary low water along that portion of the coast which is in direct contact with the open sea and the line marking the seaward limit of inland waters. . . .

This definition limits the number of land-based dischargers subject to section 403. For example, Chesapeake Bay, Boston Harbor, New York Harbor, San Francisco Bay and Puget Sound lie inside this inner boundary so that discharges into these waters are not subject to section 403 requirements. Of the approximately 62,400 existing NPDES permittees, 232 are land-based point sources discharging seaward of this inner boundary. These include 102 publicly-owned treatment works, 74 industrial plants, 25 steam electric plants and 31 federal facilities. These figures do not include the dischargers in Alaska whose location relative to the baseline defining the ocean boundary is not known.

In addition to these land-based dischargers, section 403 applies to all other point sources discharging into the marine waters covered by this regulation. By far the largest group of marine dischargers are oil and gas exploratory and production facilities. The Agency estimates that there are approximately 3,000 such facilities now operating.

III. Modifications to the Proposal

EPA provided both an oral hearing and a written comment period on the proposal, the latter extended by thirty days in response to the request of several interested groups. The preamble to the proposal specifically solicited comment on certain points, including mixing zone definition and determination, control of toxic pollutants; monitoring requirements and procedures; and effect of meeting requirements for a Section 301(h) variance.

Ten persons testified at the March 21, 1980 hearing on the proposal, and written comments were received from 81 parties, including many industrial groups, municipalities, conservation groups, federal agencies and several State governments. A listing of these commenters is presented in Appendix A. The commenters addressed the issues raised for comment in the preamble and raised a range of additional issues concerning EPA's approach in developing the proposed regulations. Detailed responses to the major public comments are presented in Appendix B.

In conjunction with the public comment review, EPA has reevaluated the proposed regulations and concluded that certain changes are appropriate. The final regulations retain the basic approach of the proposal. As in the proposal, this regulation provides that the director must determine whether a discharge will cause unreasonable degradation of the marine environment. Based on review of the numerous comments, however, EPA has made certain modifications which are intended to provide greater clarity to the permit writer, to ensure consistency in application of this regulation and to minimize burdens on the permit applicant.

Under the proposed regulation, applicants were required to submit a wide range of analyses and evaluations. Numerous commenters objected, stating that submission of this information was unnecessary, and in many cases redundant. In response to these comments, the final regulations now provide that the director may request information from the applicant but is encouraged to use other available sources, such as environmental impact statements, section 301(h) variance applications, consolidated permit applications, or EPA industrial and municipal waste surveys.

These final regulations also clarify the director's authority to issue permits where certain pre-issuance determinations relating to the effects of a discharge cannot be made. These regulations now provide that, in those cases, the discharge of pollutants may be authorized only where the director has sufficient information to make reasonable determinations regarding the potential for irreparable harm from the discharge and on the availability of alternatives. These regulations also establish certain minimum permit requirements in these cases. These requirements, identified as possible permit conditions in the proposed regulation, have been made mandatory in part in response to comments that the regulations needed to provide greater guidance to the director and applicant regarding permit conditions.

Finally, with respect to the relationship between the permit requirements of section 403(c) and variances issued under sections 301(g), 301(h), or 316(a), the final regulation provides that an applicant who has met the conditions necessary to receive such a variance is presumed to be in compliance with section 403(c) for those pollutants to which the variance applies.

IV. Procedures for Issuance of Permits Under Guidelines

1. Determination of Applicability of Section 403

The threshold determination for applicability of the section 403 guidelines is whether a proposed discharge will occur seaward of the inner boundary of the territorial seas. EPA's consolidated permit regulations (45 FR 33290, May 19, 1980) require that applicants list the latitude, longitude and name of the receiving waters for each outfall. Where the director is uncertain as to whether the outfall is within the waters covered by section 403(c), he should request guidance from EPA headquarters. Where the proposed discharge is in an area where the baseline defining the boundary of the territorial seas has not been determined, EPA will request a determination from the Department of State, which is responsible for defining the boundaries of the territorial seas.

2. Determination of Information Requirements Under Section 403(c)

Once a determination has been made that section 403(c) applies to a particular discharge, the director must determine what information is required to evaluate the discharge according to the section 403(c) criteria. The first thing that the director should do is survey the

currently available information about the discharge itself and about the area in which the discharge would occur. This information would include the data contained in the consolidated application form, as well as the data available from Agency reports and studies. The director should also notify the applicant of the existence of other currently available information.

After completing this survey of the available information, the director should determine what additional information would be required from the applicant for the evaluation of the impact of the ocean discharge as required by section 403(c). The applicant will have the responsibility of collecting this additional information and of submitting it to the director.

3. Determination of Unreasonable Degradation of the Marine Environment

Section 125.121(e) defines "unreasonable degradation of the marine environment" to include—

(1) significant adverse changes in ecosystem diversity, productivity and stability of the biological community within the area of discharge and surrounding biological communities; (2) threats to human health through direct exposure to pollutants or consumption of exposed aquatic organisms; or (3) loss of esthetic, recreational, scientific or economic values which are unreasonable in relation to the benefits derived from the discharge.

Sections 125.122 (a) and (b) specify an array of factors relevant in making these determinations.

In many cases the director will be able to reach conclusions based on data related to the nature of the proposed discharge. In areas which do not contain sensitive species or unusual biological communities or are not important for surrounding biological communities, the director may conclude that discharges containing primarily conventional pollutants will not cause unreasonable degradation. This is especially appropriate where the data indicate that there will be significant mixing with the receiving waters based on the flow of the discharge and the physical characteristics of the discharge site, such as water depth and turbulence. This determination may be appropriate for such dischargers as small publicly owned treatment works ("POTWs") and for industrial dischargers such as fruit canneries and fish processors.

For discharges into areas of biological concern or for complex or toxic discharges, additional evaluation may be necessary to determine whether a proposed discharge will cause unreasonable degradation. In assessing the need for further evaluation, the director should consider the vulnerability of the area of discharge and its role in the larger biological community. Significant or sensitive areas might include spawning sites, nursery or forage areas, migratory pathways or areas necessary for other functions or critical stages in the life cycle of organisms, areas of high productivity, or areas under stress due to biological or climatic conditions or discharges from other sources. Additionally, the director should consider whether a discharge will affect marine and wildlife species which are identified as endangered or threatened pursuant to the Endangered Species Act, 16 U.S.C. § 1531 *et seq.*, and those species critical to the structure or function of the ecosystem, such as in food chain relationships.

An assessment of the potential toxicity of a discharge should initially focus on the pollutants which are present in significant quantities relative to marine water quality criteria developed pursuant to section 304(a). The potential for bioaccumulation or persistence of pollutants in the environment is of particular importance.

The director must also consider the potential impacts of the discharge on human health either directly as through physical contact or indirectly through the food chain. These factors should be addressed when considering the location of the discharge and the type and volume of the discharger's effluent.

Determinations of the economic impact of the discharge should be based on the potential effect of the discharge on such activities as commercial fishing, recreation, mineral exploitation and scientific study. In considering whether a discharge will cause unreasonable economic impacts, the social as well as economic effects on a community should be considered.

Much, if not all, of the information necessary to make these evaluations already will be available to the director. Pursuant to section 122.53 of the consolidated permit regulations, applicants for NPDES permits must submit a range of significant information, including in many cases a detailed analysis of toxic pollutants in the waste stream. Additionally, any relevant environmental impact statements or section 301(h), 301(g) or 316(a) variance applications should provide significant data about the environmental impact of the proposed discharge. EPA industrial and municipal waste surveys and any data from relevant technical and environmental field studies that may have been conducted may serve as a source of information. Finally, Coastal Zone Management Plans or proposals for designation of an area as a marine sanctuary should also contain relevant information.

In cases where available information is insufficient for the director to make a determination, he may request additional information of the applicant pursuant to § 125.124. Where further analysis of the area of the proposed discharge is required, the director may require the applicant to perform assessments similar to those identified in the Technical Support Document prepared in conjunction with EPA's section 301(h) regulations (44 FR 34784, June 15, 1979). EPA headquarters will be available to provide assistance to the permit writer in developing information requirements where the discharge may be affecting an area of biological concern.

The director should work with the applicant to determine what types of assessment are necessary and to review or evaluate the assessment as it progresses. This level of participation is intended to determine the actual feasibility and the costs of such assessments for the applicant. Furthermore, it should avoid duplicative or inadequate assessments, thereby preventing delays in permit issuance. The Agency recognizes that some of the anticipated assessments required for permit issuance on the Outer Continental Shelf are beyond those which can reasonably be expected of the applicant and will require continued Agency research efforts.

The guidelines establish a presumption that discharges in compliance with sections 301(g), 301(h), 316(a) or State water quality standards will not cause unreasonable degradation with respect to the pollutants covered by those sections. Unless available data indicate that a discharge will cause unreasonable degradation, the director need not take additional steps, including the compilation of additional data, to support a conclusion that no further limitations on the discharge of these pollutants is necessary.

4. Determination of Irreparable Harm

Section 125.123(c)(1) requires that the director determine whether a discharge will cause irreparable harm to the marine environment in situations where he cannot determine whether the discharge will cause unreasonable degradation. Although the concepts of "irreparable harm" and "unreasonable degradation" involve similar considerations, the determination of "irreparable harm" is much narrower in scope.

In assessing the probability of "irreparable harm", the director need not focus his analysis on the overall impact of the discharge. Rather, he is only required to make a reasonable determination that the discharger, operating pursuant to the permit conditions established in § 125.123(c), will not cause permanent and significant harm to the environment during the period in which further data on the effects of the discharge are collected. In cases where such data, primarily that produced by monitoring, indicate that continued discharge will produce unreasonable degradation, the discharge must be halted or additional limitations established. Although evaluation of irreparable harm may in some cases involve difficult factual issues, determinations of this kind are currently a part of the NPDES permit issuance process. Pursuant to 40 CFR 124.60(a)(2)(ii), the presiding officer at an evidentiary hearing may authorize a facility to commence discharging prior to receipt of a final NPDES permit if the permit applicant demonstrates, among other things, that the discharge will not cause "irreparable harm to the environment . . ." This is essentially the same finding which the director must now make pursuant to these guidelines.

Certain factors are particularly significant in assessing the likelihood of "irreparable harm". Two such factors are the quantity of pollutants expected to be discharged and their potential for persistence in the marine environment. For example, a permit writer might authorize the operation of exploratory oil and gas facilities or a limited number of production facilities based on a conclusion that these limited operations will not cause irreparable harm to an area.

An additional factor is the sensitivity of the area into which the discharge is proposed. The director might conclude that a discharge could cause irreparable harm to unusual and interdependent communities, such as the coral reefs and associated communities of the Flower Garden Banks proposed marine sanctuary in the Gulf of Mexico. In such areas special conditions, including the prohibition of discharge, might be required.

Finally, data on the effect of similar discharges in similar areas is directly relevant to the determination of irreparable harm. Information demonstrating the recovery of the environment after the cessation of discharges from similar facilities would be a strong indication that irreparable harm is not likely to occur. For example, data indicate that even in areas of biological concern, biological communities reestablish themselves after the termination of discharges from publicly-owned treatment works. Thus, where the other provisions of § 125.123(c) are satisfied, the director might properly conclude that discharges from POTWs pursuant to this section may be authorized while further information is being collected.

5. Determination of Reasonable Alternatives

These guidelines establish two bases for determining whether reasonable alternatives to the proposed discharge exist. The first is the physical availability of alternative sites for disposal of pollutants. Such alternative sites might include either disposal facilities located on land, discharge point(s) within internal waters, or existing ocean dumping sites approved pursuant to the Marine Preservation, Research and Sanctuaries Act. In determining whether a site is a reasonable alternative to on-site disposal, the director should consider its distance from the site of the proposed discharge and whether its use would cause unwarranted economic impact on the discharger. For example, shipping wastes over long distances would likely impose such impact. This provision is intended to ensure some rule of reason in evaluating alternatives, it is not intended to impose a "cost/benefit" analysis of alternative sites.

In considering the availability of alternatives the director shall consider, based on available information or that requested from the applicant, the estimates of the amount of material requiring disposal. He should review the availability of existing land-based disposal sites and ocean dumping sites within a reasonable distance from the point of discharge and the estimated uncommitted capacity of such sites. The director should evaluate any reports of economic impact of discharge alternatives as may be supplied by the applicant.

The second basis for evaluating the feasibility of alternative sites relates to the relative environmental harm of disposal. Pursuant to section 121(e)(2), alternative disposal sites are not considered "reasonable alternatives" if on-site disposal is judged to be environmentally preferable. Thus, the discharge of pollutants might be authorized where disposal in alternative sites might produce equal or greater environmental harm than on-site discharge, or where transportation to alternative sites might produce a significant risk of greater environmental harm or a significant risk to human safety. For example, during certain seasons it may be undesirable to transport wastes off-site in areas of the North Atlantic or Alaska. Where the environmental or human health risks of transportation are significant, such transportation should not be considered a reasonable alternative.

6. Determination of Permit Conditions

Section 125.123(d) identifies specific permit conditions which are required for the issuance of a permit where a pre-permit issuance determination regarding degradation of the marine environment cannot be made. The director may also require any necessary permit conditions identified in section 125.123(d) to assure that unreasonable degradation of the marine environment will not occur under § 125.123(a).

(a) Limiting Permissible Concentration Requirements—Section 125.123(d)(1) requires, if a determination regarding unreasonable degradation cannot be made, that the discharge must pass certain bioassay-based requirements similar to those of EPA's ocean dumping regulations (40 CFR Part 227).

The applicant must demonstrate that his discharge will not exceed the limiting permissible concentration ("LPC") at the boundary of the mixing zone for a liquid phase and a suspended particular phase bioassay, in accordance with procedures for determining the LPC which are described in *Bioassay Procedures for the Ocean Disposal Permit Program*, U.S. EPA 600/9–78–010 March 1978 and in *Ecological Evaluation of Proposed Discharge of Dredge Material into the Ocean Waters*, EPA/Corps of Engineers, July 1977. If these manuals are revised in the future, bioassays shall be performed in accordance with any such revisions.

These regulations require an LPC which is derived from, but not identical to, the ocean dumping bioassay requirements. First, these regulations do not use section 304(a)(1) marine water quality criteria as a basis for determining an LPC. By use of a bioassay-based LPC, the ocean discharge criteria address the impact of the whole effluent and account for any synergistic or antagonistic effects. EPA recognizes that section 304(a)(1) criteria may in some cases require changes to reflect site-specific conditions, and the Agency is reevaluating the use of marine water quality criteria in the ocean dumping program.

The ocean discharge criteria also use a mixing zone extending laterally 100 meters in all directions from the discharge point(s) or to the boundary of the zone of initial dilution as calculated

by a plume model approved by the director, whichever is greater, unless the director determines that the more restrictive mixing zone or another definition of the mixing zone is more appropriate. In calculating the dilution at the boundary of the mixing zone, the discharger may use any of the various documented plume models and dispersion models appropriate for the discharge and approved by the director. Some of these models are referenced in the technical documents for EPA's ocean dumping regulations and the technical document for EPA's section 301(h) regulations.

Where the discharge contains a solid phase, the applicant will be required to perform the solid phase bioassay and bioaccumulation testing on the waste material in accordance with procedures described in *Ecological Evaluation of Proposed Discharge of Dredge Material into the Ocean Waters*. EPA/Corps of Engineers, July 1977. For example, if a bioassay analysis is required in the case of offshore oil and gas platforms, the solid phase bioassay would be conducted as a test on drilling muds and cuttings which are to be discharged.

Not all applicants may have to perform bioassay tests on their effluents. Applicants may submit bioassay analyses performed on other wastes if the applicant provides documentation to show that the composition of the waste analyzed typifies that which the applicant is discharging or intends to discharge.

(b) Monitoring Requirements—Where a pre-issuance determination regarding degradation of the marine environment cannot be made, § 125.123(d)(2) requires that a monitoring program be in place which is sufficient to assess the impact of the discharge on water, sediment, and biological quality including, where appropriate, analysis of the bioaccumulative and/or persistent impact on aquatic life of the discharge. This monitoring program may include effluent analysis, bioassay analysis and field studies. The technical document for EPA's section 301(h) regulations should provide support in developing such a monitoring program. It is not possible to make an *a priori* determination as to what constitutes an acceptable monitoring program. Site-specific conditions such as the size of the discharger's waste stream, the types of pollutants discharged, and the location of the discharge will play a role in determining what if any specific monitoring will be required under section 403(c) in addition to other NPDES monitoring requirements.

Section 125.123(d)(2) provides the director a flexible mechanism to develop such site-specific monitoring requirements. For example, a low volume discharger whose waste stream is unlikely to contain significant amounts of toxic pollutants will not be required in most cases to establish a monitoring program under these regulations. Similarly, a discharger of pollutants into an area of biological concern may be subject to more stringent monitoring requirements than one not discharging into such an area.

Monitoring programs, in some instances, may be coordinated for several dischargers. For example, with offshore oil and gas platforms, areawide monitoring programs for several dischargers may be the desirable monitoring approach. EPA headquarters has been active in assisting the regions in developing monitoring programs for offshore oil and gas exploration in areas of biological concern such as the Flower Garden Banks and Georges Bank. Those monitoring programs will serve as valuable guides for the development of additional monitoring programs for other areas of offshore oil and gas exploration and production. EPA headquarters will continue to play an active role in providing technical assistance in developing such monitoring programs.

(c) Other Permit Conditions—Under § 125.123(d)(3), the director may also require under other permit conditions on the discharge. For example, the director may require seasonal restrictions on the volume of wastes discharged where such restrictions are needed to assure protection of the marine environment. Seasonal restrictions may be necessary where the discharge is itself affected by seasonal conditions or where the biological community may become more sensitive to the impact of the discharge during certain seasonal conditions, such as during migration or spawning.

The director may require that the applicant perform bioaccumulation testing of the liquid and/or suspended particulate phase of the discharge where the director suspects such potential for bioaccumulation may exist, based on the nature of the pollutants discharged.

The director may also require process modifications, such as the substitution of less hazardous chemicals for those which are potentially harmful. He may also require process changes which would favor the recycling and reuse of potentially harmful pollutants. The Agency has recently established a task force to evaluate the discharges from offshore oil and gas exploration and production facilities and to evaluate alternate control strategies to mitigate the effects of such discharges, which include drilling muds and cuttings and produced water. Its recommendations may be used in drafting future requirements under section 403(c) authority.

The director may also require that diffuser systems for the discharger be sufficient to assure adequate dispersion of the waste stream.

7. The Administrative Process and Burden of Proof

Under the Act and this regulation, the director is responsible for making "reasonable judgments" on the preceding issues, and these judgments will be made on available information compiled in the administrative record of the permit issuance. As discussed above, this information may come from many sources including data submitted under the consolidated permit application form, environmental impact statements or section 301(h) variance applications. These guidelines do not require that all applicants submit specific information to support the section 403 determinations, and the director is encouraged to make use of existing information not prepared by applicants.

However, under the Clean Water Act, the Administrative Procedure Act, and EPA's consolidated permit regulations it is the applicant who is responsible for persuading the Agency that a permit should be issued. See 40 CFR 124.85(a)(1) and Opinion of the General Counsel No. 72. This obligation is particularly apparent with respect to applicants seeking permits to discharge into marine waters. Section 403(c)(2) requires that the director deny an NPDES permit application if there is insufficient information to make reasonable judgments under the guidelines. This means that the permit applicants should be prepared to submit sufficient information to support a determination to issue an NPDES permit.

Under the Agency's permit issuance procedures there is opportunity to submit information for the administrative record. An applicant or interested person who disputes any permit condition or tentative decision to deny an application must submit available information supporting their position during the public comment period. 40 CFR 124.13. In any subsequent evidentiary hearing on the permit, the Agency will have the burden of going forward to present its case supporting a challenged permit condition, but, at the conclusion of the Agency's presentation, the applicant or any other hearing participant has the burden of going forward to present its case. 40 CFR 124.85(a) (2) and (3). Moreover, the ultimate burden of persuading the

Agency to issue a permit remains at all times on the permit applicant.

V. Cost and Economic Impacts

Executive Order 12044, 43 FR 12661 (March 23, 1978), requires EPA and other agencies to perform Regulatory Analyses of certain regulations. EPA's plan for implementing Executive Order 12044, 44 FR 30988 (May 29, 1979), requires a Regulatory Analysis for major regulations involving annual compliance costs of $100 million or meeting other specified criteria. Where these criteria are met, EPA's implementation plan requires a formal Regulatory Analysis including an economic impact analysis and an evaluation of regulatory alternatives. The Agency has determined that none of the criteria for requiring a regulatory analysis has been exceeded and therefore, the promulgated regulations for ocean dischargers do not require a formal Regulatory Analysis. Nevertheless, EPA performed an analysis that does meet all the requirements of Executive Order 12044 and EPA's plan for its implementation.

In accordance with the requirements under section 2(d)(8) of Executive Order 12044, the Agency intends to review the effectiveness and need for continuation of the provisions contained in this action no more than five (5) years from the effective date of these regulations. In particular, we will solicit comments from affected parties with regard to actual costs incurred and other burdens associated with compliance and will also review environmental data to evaluate the effectiveness of the regulation after it has gone into effect.

EPA's economic analysis divides the affected dischargers under the proposed regulation into five subcategories: POTWs, industrial dischargers, electric utilities, federal facilities, and offshore oil and gas wells. This analysis has assessed unit price increases, production changes for industrial dischargers, and user cost increases at POTWs.

The total cost of compliance is expected to be $13 million in 1981 and to increase to $28 million by 1985, in 1980 dollars. Costs may increase in succeeding years. The Agency expects no significant economic impacts will result from this regulation.

1. POTW s,

There are presently 102 POTWs discharging 2.1 billion gallons of effluent a day into the ocean. These POTWs are currently operating under EPA's NPDES regulations. Under these regulations POTWs may be required to monitor, perform benthic analyses, bioaccumulation tests and run further analyses of disposal alternatives in addition to those required under their present NPDES permit. The Agency performed an economic analysis to determine the potential costs and increases in user charges currently paid by households serviced by affected POTWs. EPA estimates that 47 POTWs will incur additional costs, due to their location and the size of their discharges, consisting of a first year cost of $1.2 million and an average annual cost of $.828 million thereafter.

For 46 of the 47 affected POTWs, user charges will increase between $.09 to $.83 per family per year. The impact analysis then compared these costs to median family incomes and found that no significant economic impacts would occur. However, the impact analysis has indicated that for one community user costs could increase by $33.00 per family per year.

Currently 36 POTWs subject to these regulations have applied for 301(h) variances. EPA has not yet begun to issue decisions on section 301(h) variance requests. However, much of the information generated for purposes of section 301(h) applications can be utilized in determining compliance with the requirements of these regulations. Furthermore, in recent years there has been a trend towards centralization of POTWs in many coastal areas. This continued centralization will reduce the number of ocean outfalls, thus lowering total monitoring and user costs.

2. Industrial Dischargers

Industrial dischargers will face the same type of compliance requirements as POTWs. Monitoring and compliance requirements for industrial dischargers are dependent on the particular geographic area as well as the composition and volume of the effluent. At the present time there are 74 industrial operations affected by this regulation, discharging approximately 212 million gallons per day of effluent. The Agency expects that small industrial plants discharging non-toxic pollutants will not be affected by this regulation. EPA estimates 46 dischargers will incur additional costs due to this regulation, with a first year cost of $4.72 million, and annual costs of $3.52 million in the following years.

An analysis was conducted for a sample of industrial dischargers on both the East and West Coasts to determine the potential price increases that could result due to this regulation. EPA estimates that average unit prices for products produced will generally increase less than .1 percent to comply with this regulation. No plant closures, unemployment or other significant economic impacts are expected due to these requirements.

3. Federal Facilities

At the present time there are 31 federal facilities affected by this regulation. These facilities are discharging approximately 124 million gallons per day of effluent into the ocean. The greater part of the total, 75 million gallons per day, originates from the strategic oil reserve construction site on the Texas coast. The remaining 49 million gallons a day are from a variety of small sources, e.g., Defense Department and Coast Guard installations. The Agency estimates that four federal facilities discharging 102 million gallons per day will actualiy incure additional costs from this regulation. The total annual costs for compliance under the proposed regulation is expected to be approximately $.476 million, with the largest proportion of this amount being related to the construction of the United States strategic oil reserve. EPA does not expect any significant economic impacts to occur due to expenditures by these facilities.

4. Electric Utilities

The Agency does not expect any significant costs to be incurred by electric utilities. Compliance with the present effluent limitation requirements and with regulations implementing section 316(a) of the Clean Water Act are expected to result in compliance with requirements in this regulation. The Agency expects that monitoring for chlorine discharges may be required at some facilities. However, the cost of such monitoring would not be significant, and no economic impacts are expected to occur.

5. Offshore Oil and Gas Operations

There are presently fewer than 30 oil and gas platforms which are expected to incur additional costs due to this regulation. The Agency estimates that 7,582 exploratory and production wells will be drilled between 1981–1985 with approximately 835 (11 percent) expected to incur additional costs resulting from compliance with this regulation. The Agency has based its assessment on the assumption that compliance with applicable NPDES permit requirements will generally result in compliance with these regulations for all oil and gas wells except those located in areas of biological concern. Wells that cannot meet the requirements of this regulation through compliance with their NPDES permit terms will be required to initiate

monitoring, testing, or changes in their discharge practices.

The Agency's evaluation of the economic effects of this regulation assumed the installation of "zero discharge" technologies in order to evaiuate the maximum possible impact of these regulations. The cost of "zero discharge" varies according to geographic location, differences in weather conditions, water depths, biological communities, and other similar factors. The economic analysis groups wells into four regions—the Atlantic Ocean, Gulf Ocean, West Coast, and Alaskan waters.

The number of wells that would need to make expenditures beyond those required under existing NPDES permit requirements was estimated from Department of Interior data regarding current and future lease tracts in the ocean and from current trends in new drilling. The estimates here project acuvity from 1981–1985. Should the amount of new drilling increase or decrease beyond that time the annual cost of this regulation would increase or decrease proportionately.

It is estimated that the annual cost for offshore oil and gas operations locating in or near areas of biological concern will incur compliance costs ranging from seven to 23 million dollars per year between 1981 and 1985. Costs for various forms of monitoring are expected to be less. There are numerous alternatives which include process changes, mud substitution and shunting as potential compliance alternatives. A number of combinations are possible, depending on geographic location, water depth, temperature and specific biological life. For this reason only the worst case, "zero discharge" requirement is presented here.

The typical compliance cost per year for each geographical area for the no discharge alternative is presented below. Costs for operations in the Atlantic Ocean are expected to range between .6 and 6.9 million dollars per year, and Gulf of Mexico operations will face costs ranging between 1 and 3.6 million dollars per year. Operations located on the West Coast will face costs ranging between 4.3 and 12.4 million dollars per year. However, the California Ocean Plan requires outer continental shelf operations to conform to strict State requirements, which may reduce the incremental compliance costs under this regulation. Alaskan operations will face compliance costs ranging between 1.2 and 4.5 million dollars per year.

Oil and gas prices at the well head are not expected to be affected by this regulation, since compliance costs cannot be directly passed forward due to various price controls. However, the cost of this regulation may be manifested in reduced bids for new lease tracts. The net effect of this regulation would be a loss in future revenues to the federal government in the amount which this regulation costs the oil industry.

Dated: September 26, 1980.

Douglas M. Costle,

Administrator.

Appendix A—Public Comments

The following parties responded with comments regarding the February 12, 1980 Ocean Discharge Criteria postmarked on or before the April 28, 1980 close of the public comment period: Charles A. Lunsford, Commonwealth of Virginia, State Water Control Board; State of Hawaii, Dept. of Health; County of San Diego, Community Services Agency, Dept. of Sanitation & Flood Control; City of Los Angeles, California Dept. of Public Works; Menasha Corporation; National Manufacturing Company; Commonwealth of Virginia, State Water Control; County Sanitation Districts of Los Angeles County; Crown Zellerbach Environmental Services; Kaiser Aluminum & Chemical Corporation; Boise Cascade, Paper Group; Davies Hamakua Sugar Company; Dept. of Health, Education & Welfare, Public Health Service; Hawaiian Sugar Planters' Association; Hilo Coast Processing Company; Netarts-Oceanside Sanitary District; International Paper Company; State of Alaska, Dept. of Fish & Game; Commonwealth of Virginia, Hampton Roads Sanitation District; Marathon Oil Company, Production Operations; San Francisco Wastewater Program, City and County of San Francisco, California; U.S. Cape May County Municipal Utilities Authority, New Jersey; Dept. of the Army, South Atlantic Division, Corps of Engineers; State of California, Resources Agency, Dept. of Fish and Game; National Fisheries Institute, Inc., Natural Resources Defense Council, Inc., Sussex County Council, Georgetown, Delaware; American Paper Institute/ National Forest Products Association, Environmental Program, Houston Audubon Society; Texas Eastern Transmission Corporation; State of Delaware, Department of Natural Resources and Environmental Control, Division of Environmental Control; Department of the Air Force, Engineering and Serivce Center; Star-Kist Foods, Inc.; State of California, Resources Agency, State Water Resources Control Board; Office of the Assistant Secretary of Defense, Energy, Environment and Safety; the Ocean County Utilities Authority, New Jersey, National Wildlife Federation; Department of the Army, Office of the Chief of Engineers; Shell Oil Company; Texaco, Inc., American Cyanamid Company; Atlantic Richfield Company; E. I. du Pont de Nemours and Company, Inc.; Public Service Company of New Hampshire; Chevron U.S.A., Inc., Environmental Affairs; Commonwealth of Puerto Rico, Puerto Rico Aqueduct and Sewer Authority; Exxon Company, U.S.A.; Offshore Operators Committee, Southern California Edison Company; Virgin Islands Rum Industries, Ltd., Fried, Frank, Harris, Shriver & Kampelman; Chevron U.S.A. Inc., Pillsbury, Madison & Sutro; City of Watsonville, California; Conoco, Inc.; Conservation Law Foundation of New England, Inc.; Mobil Oil; Corporation; Western Oil & Gas Association; Alaska Lumber and Plup Company, Inc., Robertson, Monagle, Eastaugh & Bradley; American Petroleum Institute; Cody Biggs; Chemical Manufacturers Association, Covington & Burling; City of Skagway, Alaska, Robertson, Monagle, Eastaugh & Bradley; Columbia Gas System Service Corporation; Department of Energy; Gulf Oil Exploration & Production Company; National Food Processors Association; Pacific Legal Foundation; Phillips Petroleum Company; Tuna Research Foundation, Inc.; Union Oil Company of California; U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Environmental Research Laboratories; Natural Resources Defense Council, Inc.; U.S. Fish & Wildlife Service, Alaska Area Office; Utility Water Act Group, Hunton, & Williams; Department of Water & Power, the City of Los Angeles, California; U.S. Department of the Interior, Geological Survey.

The following parties responded with comments postmarked after the April 28, 1980 close of public comment period: U.S. Department of the Interior; Chevron, U.S.A., Pillsbury, Madison & Sutro; University of Southern Maine for State Planning Office, State of Maine; Monmouth County Board of Health, New Jersey; State of Maine, State Planning Office; Western Oil and Gas Association.

The following parties testified at the March 21, 1980 hearing: George P. Haley, Chevron USA; Frank Parker, Coordinator, Environmental and Government Affairs Chevron USA; Elizabeth F. Kroop, Counsel for the National Wildlife Federation; Walter J. Zizik, Project Coordinator, South Monmouth Regional Sewerage

Authority; Curt D. Rose, Manager, Aquatic Sciences Division, Energy Resources Company; Frank Melone, Southern California Edison Company; William A. Anderson, Attorney, Utility Water Act Group; Joseph F. Dietz, Coordinator of Environmental Affairs, San Diego Gas and Electric Company; Edward G. Gladbach, Civil Engineer, Department of Water and Power, City of Los Angeles; Peter Holmes, Research Assistant, Atlantic Coast Project, Natural Resources Defense Council.

Appendix B—Response to Public Comments

1. *Comment.* The Agency received several public comments questioning the accuracy of the inventory in the proposal and noting that there was uncertainty over the exact location of the baseline marking the landward boundary of the territorial seas, particularly in parts of Alaska, Florida, Puerto Rico, Oregon and Washington.

Response. Even where the baseline has not been plotted, there are available nautical charts for the various bays and harbors in question. The Office of the Geographer in the Department of state is responsible for the charts plotting closing lines across islands and shoreline markers depicting the baseline of the territorial sea. That office is assisted by the Interagency Baseline Committee, chaired by the Department of State, with other members coming from the National Oceanic and Atmospheric Administration, the Coast Guard, and the Departments of Justice and the Interior. The Committee meets several times a year to make baseline determinations.

To facilitate the ongoing implementation of section 403, EPA has identified dischargers whose coverage under that provision is in question, due to uncertainty concerning the location of the baseline. The Agency has submitted a written rquest to the Department of State and the Interagency Baseline Committee for a determination whether these dischargers are outside the baseline of the territorial seas and thus subject to section 403. EPA will continue to seek determinations when NPDES permits are issued, modified, or reissued, where there is doubt as to whether a discharger is within the purview of section 403.

2. *Comment.* One commenter stated that while it appeared that the Agency intended for coastal electric utilities to be subject to the regulations, these plants were not counted among the 71 land-based industrial dischargers.

Response. Electric utilities outside the baseline are covered by this regulation and EPA, in response to the comment, confirmed this with representatives of the affected industry during the public comment period. The Agency also extended the comment period by thirty days, at the request of this commenter and others, to allow additional time for submission of comments on the proposal.

The Agency has identified 25 covered plants, which are included in the cost and economic impact analysis for the regulation. In addition, EPA has updated the inventory of subject marine dischargers. As the preamble notes, the number of land-based dischargers subject to section 403 is limited. The updated inventory identifies 232 such dischargers, including 102 POTWs, 74 industrial facilities, 25 steam electric utilities, and 31 federal facilities. These figures do not include dischargers in Alaska whose location relative to the baseline defining the boundary of the territorial seas has not been established. However, the Agency believes that most of these dischargers are small and that any environmental or economic impacts would be minimal. The Agency also estimates that there are some 3,000 subject offshore oil and gas platforms.

3. *Comment.* A number of commenters stated that under the proposed regulation, ocean dischargers might be subject to more stringent controls and, accordingly, might incur higher costs than would dischargers into potentially more sensitive estuarine and freshwater systems where the assimilative capacity of the body of water may be less than in the oceans and the potential impact of pollution on the ecosystem greater.

Response. As the preamble to the regulation notes, the Clean Water Act limits the coverage of section 403 to dischargers into waters seaward of the baseline marking the territorial seas. This additional assurance of protection and its limitation to the waters of the territorial seas, the contiguous zone, and the oceans is, therefore, a matter of Congressional mandate. EPA has designed its regulations to provide this protection, as Congress has directed. As to the question of costs, the Agency anticipates that in most cases, technology-based effluent limitations required under other provisions of the Act will be adequate in themselves to afford the necessary protection for the marine environment. As to freshwater and estuarine systems, the Agency agrees that these waters must be protected also; the statutory authority to accomplish this, however, rests in other sections of the Act and in other environmental statutes.

4. *Comment.* A number of commenters suggested that the ocean discharge criteria should merely have the effect of guidelines, rather than regulatory requirements, and should provide flexibility and allow for discretion on the part of the director to apply appropriate portions of the guidelines to the situation of an individual discharger.

Response. As noted in the preamble, these regulations, although from time to time described as "guidelines" or "criteria" to avoid repetition, establish mandatory requirements authorized by section 403(c). Whatever the terminology, they have the effect of mandatory regulations because, at any time that promulgated guidelines are in effect, no NPDES permit may be issued "except in compliance with such guidelines." Nevertheless, the regulation provides the permit writer flexibility in tailoring information requests and permit conditions to the circumstances of individual dischargers, based on local conditions.

5. *Comment.* Several commenters expressed the concern that while the proposed regulation required a permit applicant to demonstrate to the director that its discharge would have no unreasonable adverse impact on the environment, or that adequate toxics control and monitoring programs were in place, the proposal failed to tell the applicant how to make such a demonstration.

Response. In order to ensure that applicants will receive adequate guidance, the final regulation has been clarified to require that the director inform an applicant of any specific information that must be supplied. In addition, in an attempt to minimize the information collection obligation of applicants, the final regulation provides that the director may consider information already available to him in making the determinations required under § 125.123(a), (b), or (c).

6. *Comment.* Several commenters suggested that, for the territorial seas, the purposes of section 403 were being served already by State water quality standards required under section 303 and by technology-based effluent limitations under sections 301 and 304 of the Clean Water Act. Another commenter stated that pretreatment programs required under section 307 should also ensure protection of the marine environment.

Response. The Agency agrees that State water quality standards, technology-based limitations and pretreatment programs are all necessary to protect the marine environment. While in most instances discharges in compliance with such standards, limits, and programs will also be determined to pass the "no unreasonable degradation" test in these regulations, there may be

instances where this will not be the case. For example, there may be instances where no State water quality standards have been established for specific pollutants being discharged. Further, State water quality standards do not generally apply beyond the limits of the territorial seas, while the section 403 criteria apply also to the contiguous zone and the oceans. In addition, there may be instances in which technology-based controls will not be sufficient to assure protection of a particular marine environment, necessitating more stringent controls to assure that the section 403 criteria are met. Such may also be true of pretreatment programs; while the director may consider the effectiveness of a given pretreatment program in making the "unreasonable degradation" determination, he should not assume that the existence of pretreatment ensures protection of the marine environment for purposes of section 403.

7. *Comment.* Several commenters stated that while the proposed regulation differentiated special "areas of biological sensitivity" to assure those areas were not adversely affected, the regulation failed to adequately define what constitutes such areas. Another commenter suggested that the term should be replaced by "areas of biological concern" because "biological sensitivity" connotes a narrow concern for unique or fragile ecosystems. The commenter suggested that the emphasis of the regulation should be on unwarranted ecological damage regardless of the biological sensitivity of the area.

Response. The scope of this regulation is broader than protecting only those areas that are termed "sensitive"; as in the proposed regulation, these guidelines seek to prevent unreasonable degradation of the marine environment regardless of where the discharge occurs. Although the regulation no longer attempts to classify areas as "sensitive" or "nonsensitive", the location of the discharge is an important element in determining the level of control necessary to prevent such degradation. Section 125.122 identifies for the director a number of factors relating to the biology of the local community which are important in assessing the impact of a discharge.

8. *Comment.* A substantial number of comments submitted on behalf of various dischargers suggested that the dischargers in question—including small POTWs, electric utilities, seafood processors, Alaskan logging operations, and offshore oil and gas exploration and production wells—should be exempt from the requirements of section 403. Several commenters made the argument, in some cases based on submissions of technical data and reports, that their discharges already were subject to controls adequate to protect the marine environment. Some stated that their discharges resulted in only *de minimus* effects on the environment. Some stated that compliance with various provisions of the proposal would result in economic hardship.

Response. EPA has concluded that there is no basis for categorically exempting classes of subject dischargers from the coverage of section 403. While the data submitted by some commenters may be useful in determining whether a particular discharge will meet the "unreasonable degradation" test, it does not provide a basis for such a blanket exemption. However, while a permit writer is not precluded from seeking additional site-specific information, the submission of large quantities of data for particular dischargers or classes of dischargers makes it unlikely that a permit writer will find it necessary to require these applicants to submit any substantial quantity of additional data. Similarly, in the cases of small POTWs and others where the discharge is expected to have only a minimal impact, the flexibility which the final regulation provides will allow the permit writer to take this situation into account, rather than mandating a rigid across-the-board application of all requirements, with their associated costs.

9. *Comment.* Several commenters suggested that POTWs granted section 301(h) variances from secondary treatment requirements should be exempt from section 403 because of significant similarities in the two provisions. Another commenter, however, stated that section 301(h) contains no analogue to section 403(c)(1)(F) or (G) and asserted that toxic pollutants are not adequately controlled under section 301(h).

Response. Despite differences in statutory language, sections 403 and 301(h) share similar objectives in seeking to assure protection of the marine environment, and the respective determinations whether those objectives have been met under each provision is based on similar information. Section 301(h)(2) requires that a successful applicant for a variance demonstrate, among other things, that "such modified requirements will not interfere with the attainment or maintenance of that water quality which assure protection of public water supplies and the protection of shellfish, fish, and wildlife, and allows recreational activities in and on the water." Section 125.61 of EPA's section 301(h) regulations requires full and detailed descriptions of the physical characteristics of the discharge, its biological impact on the marine environment, and its impact on public water supplies and recreation. Given therefore that a successful section 301(h) applicant will have collected and presented substantial amounts of data on the effect of its discharge on the marine environment, including its inhabitants and uses, the final ocean discharge regulations provide that a successful section 301(h) demonstration creates a rebuttable presumption that an applicant will satisfy the section 403(c) guidelines as well. While a permit writer is not precluded from placing additional requirements on such an applicant under these regulations, it is unlikely that this will be necessary in light of the through-going demonstration the applicant has made for purposes of section 301(h).

This approach is consistent with legislative history to the effect that section 301(h) applicants must comply also with section 403. This language indicates that Congress did not intend for section 403 to become a dead letter with the subsequent enactment of section 301(h). Unlike the approach of those commenters who sought to make compliance with section 403 automatic for an applicant who had obtained a section 301(h) variance, the "rebuttable presumption" approach does not treat section 403 as redundant. Nor, however, does it impose a redundant data-gathering task on successful section 301(h) applicants either, taking account as it does of the unmistakable similarities in the showings required under the two provisions.

The Agency disagrees with the comment that the toxic control provisions of the section 301(h) regulations are not adequate. Moreover, if a permit writer determines that toxic pollutants in the discharge of a successful section 301(h) applicant are not adequately controlled for purposes of section 403, he can require additional controls or, if necessary, require zero-discharge permit terms for those pollutants.

10. *Comment.* Several commenters suggested that coastal steam electric utility plants granted a variance under section 316(a) of the Act should be exempt from demonstrating compliance with section 403, on the grounds that the demonstration necessary for obtaining a section 316(a) variance provides the requisite assurance that the marine environment is protected for purposes of section 403(c).

Response. To obtain a section 316(a) variance, an applicant must demonstrate

that effluent controls on its thermal discharge will be sufficient to assure the protection and propagation of a balanced indigenous population of shellfish, fish and wildlife in and on the water. The Agency agrees that in most cases the demonstration required of a successful section 316(a) applicant will be sufficient to allow the permit writer to conclude that there will be no unreasonable degradation of the marine environment due to excess heat. While on the reasoning set out in the previous response successful section 316(a) applicants will not be exempt from section 403, the regulation provides that a successful section 316(a) application creates a rebuttable presumption of compliance with section 403(c) for the thermal component of the discharge.

11. *Comment.* Commenters also suggested that those publicly-owned treatment works which installed secondary treatment should be exempt from requirements under section 403.

Response. Limitations established pursuant to section 403 are a supplement to technology-based limitations such as secondary treatment for POTWs, and no class of a discharger is exempt from compliance with these regulations. However, it is likely that secondary treatment will generally be adequate to satisfy section 403 requirements where there is adequate pretreatment by industrial sources and where the POTW is not discharging into areas of biological concern.

12. *Comment.* As noted above, some commenters asserted that the ocean discharge criteria should merely be guidelines providing information to the permit writer. Other commenters, however, stated that the guidelines should require that a discharge pass a quantitative test, such as the bioassay requirements used in the ocean dumping regulations, and comply with State and EPA water quality criteria as a prerequisite to permit issuance.

Response. The Agency has revised the proposed regulation to allow necessary flexibility to the director in assessing both the impact of a discharge and permit limitations. However, the regulation does impose minimum permit limitations, including a bioassay-based limitations, in areas where the long range impact of a discharge is not fully understood. This approach should provide certainty and consistency in permit limitations in areas where the determinations by the permit writer would be the most difficult and complex.

Discharges into the territorial seas must comply with any applicable state water quality criteria. However, the Act generally does not provide for the application of these criteria to the contiguous zone and oceans. Although the Act establishes a complete water quality program for State waters based on designated uses and supporting criteria, no such scheme exists for marine waters beyond State jurisdiction. The 403(c) regulation is consistent with the Agency policy outlined in the section 301(h) regulations (44 FR 34810–34811), and will utilize water quality criteria published pursuant to section 304(a)(1), as they are developed, as a basis for assessing the environmental impact of such pollutants.

13. *Comment.* Several commenters asserted that, under the proposed regulation, no predischarge determination was required by the director to assure that the marine environment was protected. Instead, commenters stated, the proposed regulation relied on post discharge monitoring.

Response. Under the final regulations, no discharge of pollutants may be authorized unless, before permit issuance, the director has sufficient information to make a reasonable determination that there will be no irreparable harm to the environment while monitoring is undertaken to determine if there will be unreasonable degradation. In addition the permit must specify certain mandatory limitations.

14. *Comment.* The Agency received numerous comments regarding the monitoring requirements outlined in section 125.127 of the proposed regulation. The major issue raised was that the monitoring requirements should be as flexible as possible providing an applicant a clear description of the information he must provide. Commenters suggested that the rigor of the monitoring program should be tailored to site-specific conditions such as the nature and location of the discharge. In addition, a number of commenters stated that compliance with the proposed monitoring requirements would result in severe economic hardship for small dischargers. It was suggested that the latter, especially small POTWs, be exempted from the monitoring requirements.

Response. As discussed in the preamble, these regulations have been revised to allow the permit writer to request from the applicant only that information necessary to make judgments required by the guidelines. In some cases this will involve monitoring programs, and the director will work with the applicant in identifying specific information that must be supplied as part of the permit application process.

Since no discharge may be allowed which would result in unreasonable degradation of the marine environment, and since the permit writer must be afforded the means to make the necessary determinations under these regulations, EPA has concluded that it may not exempt categories of dischargers, even small dischargers, from monitoring requirements as an initial matter. Nevertheless, the final regulations do not require monitoring in all cases, and, where monitoring is necessary, provide for flexibility in fashioning site specific requirements. Although any monitoring that may be necessary will depend on the nature and location of the discharge in question, small dischargers generally are not expected to incur significant economic costs as a result of this regulation.

15. *Comment.* Several commenters suggested that, in light of the similarities between section 403(c) of the Clean Water Act and section 102(a) of the Marine Protection, Research and Sanctuaries Act, the ocean discharge criteria should be similar to the ocean dumping regulations.

Response. EPA recognizes that in section 403(c) of the Clean Water Act and section 102(a) of the Marine Protection, Research, and Sanctuaries Act, Congress adopted similar although not identical provisions. Hence, in the regulations implementing the respective statutes, similar criteria may be appropriate.

EPA first promulgated ocean dumping criteria in 1973; those criteria were amended in 1977. Initially, the regulations served as joint regulations for the CWA and the MPRSA. Since promulgation of the ocean dumping regulations, however, EPA has received a number of comments based on those regulations. In addition, increasing data has become available in respect to the environmental impact of disposing of material at various locations in the ocean, by various methods.

The ocean discharge regulations being promulgated today are based on the latest data and information available to EPA, and the Agency believes these regulations are consistent with the CWA and with current scientific and technical knowledge. Various factors, including the MPRSA comments and the new data, suggest that it may now be appropriate for EPA to review the ocean dumping regulations as well. Such a review may provide further insights on an appropriate overall approach for protecting the ocean; and inconsistencies which may exist between the current sets of regulations can be resolved in the context of that action. However, in addition to any statutory distinctions, differences in the manner of disposal and the types of pollutants discharged may warrant

different regulatory approaches under these two statutes.

16. *Comment.* Several commenters suggested that dischargers who were not causing unreasonable degradation of the marine environment should not be required to assess the availability of alternatives.

Response. The proposed regulation has been modified to require the assessment of reasonable alternatives only where the director cannot determine whether the discharge will cause unreasonable degradation of the marine environment. In cases where the director determines that the discharge will not cause such degradation, an NPDES permit may be issued notwithstanding the availability of an alternative to ocean disposal.

Although the Clean Water Act contains as an ultimate goal the complete elimination of the discharge of pollutants, the water quality provisions of the Act, including sections 303 and 403, do not require that discharges into either inland or offshore waters be prohibited in the absence of unreasonable water quality impacts.

17. *Comment.* Several commenters expressed concern that the requirements for general permits, specified in section 125.129 of the proposed regulation, were not consistent with the requirements for individual permits.

Response. Section 125.129 has been deleted from the final regulation, and the director is required to make the same determinations when issuing either general or individual permits.

18. *Comment.* A few commenters objected to language in the preamble of the proposal to the effect that the permitting authority would be free to draw on his own knowledge of conditions in the vicinity of an outfall in determining whether a discharge adversely affected the marine environment. These commenters expressed the "due process" concern that this language allowed the permitting authority to issue permits on the basis of information not made known to the permittee and not in the administrative record.

Response. The language in question does not appear in the final regulation or in its preamble. While the regulation provides that the director make the determinations under §.125.123(a), (b), or (c) on the basis of "available information," that language was added in response to the suggestion of numerous commenters that permit applicants should not be required for purposes of Section 403 to resubmit data which was already available to the permit writer.

19. *Comment.* EPA received comment to the effect that the reference in the proposed regulations to schedules allowing additional time for compliance with Section 403 requirements should be limited to existing dischargers, consistent with the provisions of the NPDES regulations.

Response. Section 125.123(d)(3) of the final regulation provides for "schedules of compliance for existing dischargers," as suggested above.

20. *Comment.* The Agency received several comments regarding the mixing zone analysis as described in § 125.123. Some commenters suggested that the models identified by the Agency in the proposal would not be appropriate for all types of discharge. Other commenters suggested that the Agency should use the ocean dumping mixing zone definition.

Response. The mixing zone analysis in the final regulations is intended for use in calculating whether the limiting permissible concentration is violated in instances where bioassay analysis is required. The proposed regulation required a mixing zone analysis for all dischargers to assure that, following initial dilution, the discharge was dispersed so as not to adversely affect areas of biological sensitivity. As noted previously, this requirement has been deleted from the final regulation. In the final regulation, a mixing zone analysis is required only in those instances where the director cannot determine that unreasonable degradation will not occur and where a bioassay analysis is required.

The mixing zone definition in these regulations is consistent with the ocean dumping mixing zone definition identified in the EPA/Corps of Engineers technical manual, with some modifications to account for the differences in the nature of discharged wastes versus those which are dumped. The ocean dumping mixing zone was devised primarily to facilitate analysis of impacts from intermittent discharges from moving vessels, whereas the 403(c) regulations are intended to facilitate analysis of continuous discharges from stationary sources. The final regulation also allows the discharger to use alternative methods for determining the mixing zone where scientific evidence demonstrates they are appropriate and where EPA concurs.

A new Subpart M is added to read as follows:

Subpart M—Ocean Discharge Criteria

Sec.

125.120 Scope and purpose.
125.121 Definitions.
125.122 Determination of unreasonable degradation of the marine environment.
125.123 Permit requirements.
125.124 Information required to be submitted by applicant.

§ 125.120 Scope and purpose.

This subpart establishes guidelines for issuance of National Pollutant Discharge Elimination System (NPDES) permits for the discharge of pollutants from a point source into the territorial seas, the contiguous zone, and the oceans.

§ 125.121 Definitions.

(a) "Irreparable harm" means significant undesirable effects occurring after the date of permit issuance which will not be reversed after cessation or modification of the discharge.

(b) "Marine environment" means that territorial seas, the contiguous zone and the oceans.

(c) "Mixing zone" means the zone extending from the sea's surface to seabed and extending laterally to a distance of 100 meters in all directions from the discharge point(s) or to the boundary of the zone of initial dilution as calculated by a plume model approved by the director, whichever is greater, unless the director determines that the more restrictive mixing zone or another definition of the mixing zone is more appropriate for a specific discharge.

(d) "No reasonable alternatives" means: (1) No land-based disposal sites, discharge point(s) within internal waters, or approved ocean dumping sites within a reasonable distance of the site of the proposed discharge the use of which would not cause unwarranted economic impacts on the discharger, or, notwithstanding the availability of such sites,

(2) On-site disposal is environmentally preferable to other alternative means of disposal after consideration of: (i) The relative environmental harm of disposal on-site, in disposal sites located on land, from discharge point(s) within internal waters, or in approved ocean dumping sites, and

(ii) The risk to the environment and human safety posed by the transportation of the pollutants.

(e) "Unreasonable degradation of the marine environment" means: (1) Significant adverse changes in ecosystem diversity, productivity and stability of the biological community within the area of discharge and surrounding biological communities,

(2) Threat to human health through direct exposure to pollutants or through consumption of exposed aquatic organisms, or

(3) Loss of esthetic, recreational, scientific or economic values which is unreasonable in relation to the benefit derived from the discharge.

§ 125.122 Determination of unreasonable degradation of the marine environment.

(a) The director shall determine whether a discharge will cause unreasonable degradation of the marine environment based on consideration of: (1) The quantities, composition and potential for bioaccumulation or persistence of the pollutants to be discharged;

(2) The potential transport of such pollutants by biological, physical or chemical processes;

(3) The composition and vulnerability of the biological communities which may be exposed to such pollutants, including the presence of unique species or communities of species, the presence of species identified as endangered or threatened pursuant to the Endangered Species Act, or the presence of those species critical to the structure or function of the ecosystem, such as those important for the food chain;

(4) The importance of the receiving water area to the surrounding biological community, including the presence of spawning sites, nursery/forage areas, migratory pathways, or areas necessary for other functions or critical stages in the life cycle of an organism.

(5) The existence of special aquatic sites including, but not limited to marine sanctuaries and refuges, parks, national and historic monuments, national seashores, wilderness areas and coral reefs;

(6) The potential impacts on human health through direct and indirect pathways;

(7) Existing or potential recreational and commercial fishing, including finfishing and shellfishing;

(8) Any applicable requirements of an approved Coastal Zone Management plan;

(9) Such other factors relating to the effects of the discharge as may be appropriate;

(10) Marine water quality criteria developed pursuant to section 304(a)(1).

(b) Discharges in compliance with sections 301(g), 301(h), or 316(a) variance requirements or State water quality standards shall be presumed not to cause unreasonable degradation of the marine environment, for any specific pollutants or conditions specified in the variance or the standard.

§ 125.123 Permit requirements.

(a) If the director on the basis of available information including that supplied by the applicant pursuant to § 125.124 determines prior to permit issuance that the discharge will not cause unreasonable degradation of the marine environment after application of any necessary conditions specified in § 125.123(d), he may issue an NPDES permit containing such conditions.

(b) If the director, on the basis of available information including that supplied by the applicant pursuant to § 125.124 determines prior to permit issuance that the discharge will cause unreasonable degradation of the marine environment after application of all possible permit conditions specified in § 125.123(d), he may not issue an NPDES permit which authorizes the discharge of pollutants.

(c) If the director has insufficient information to determine prior to permit issuance that there will be no unreasonable degradation of the marine environment pursuant to § 125.122, there shall be no discharge of pollutants into the marine environment unless the director on the basis of available information, including that supplied by the applicant pursuant to § 125.124 determines that: (1) Such discharge will not cause irreparable harm to the marine environment during the period in which monitoring is undertaken, and

(2) There are no reasonable alternatives to the on-site disposal of these materials, and

(3) The discharge will be in compliance with all permit conditions established pursuant to paragraph (d) of this section.

(d) All permits which authorize the discharge of pollutants pursuant to paragraph (c) of this section shall: (1) Require that a discharge of pollutants will: (A) following dilution as measured at the boundary of the mixing zone not exceed the limiting permissible concentration for the liquid and suspended particulate phases of the waste material as described in section 227.27(a) (2) and (3), section 227.27(b), and section 227.27(c) of the Ocean Dumping Criteria; and (B) not exceed the limiting permissible concentration for the solid phase of the waste material or cause an accumulation of toxic materials in the human food chain as described in sections 227.27 (b) and (d) of the Ocean Dumping Criteria;

(2) Specify a monitoring program, which is sufficient to assess the impact of the discharge on water, sediment, and biological quality including, where appropriate, analysis of the bioaccumulative and/or persistent impact on aquatic life of the discharge;

(3) Contain any other conditions, such as performance of liquid or suspended particulate phase bioaccumulation tests, seasonal restrictions on discharge, process modifications, dispersion of pollutants, or schedule of compliance for existing discharges, which are determined to be necessary because of local environmental conditions, and

(4) Contain the following clause: In addition to any other grounds specified herein, this permit shall be modified or revoked at any time if, on the basis of any new data, the director determines that continued discharges may cause unreasonable degradation of the marine environment.

§ 125.124 Information required to be submitted by applicant.

The applicant is responsible for providing information which the director may request to make the determination required by this subpart. The director may require the following information as well as any other pertinent informaton: (a) An analysis of the chemical constituents of any discharge;

(b) Appropriate bioassays necessary to determine the limiting permissible concentrations for the discharge;

(c) An analysis of initial dilution;

(d) Available process modifications which will reduce the quantities of pollutants which will be discharged;

(e) Analysis of the location where pollutants are sought to be discharged, including the biological community and the physical description of the discharge facility;

(f) Evaluation of available alternatives to the discharge of the pollutants including an evaluation of the possibility of land-based disposal or disposal in an approved ocean dumping site.

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